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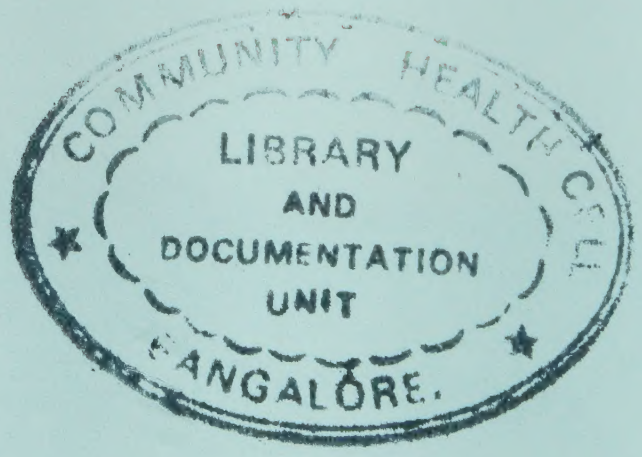

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PREFACE

Malaria situation in the country as a whole has not changed. In some areas there have been a spurt and in others decline, but this is due to unstable nature of malaria. The problem of drug resistance in *P. falciparum* is increasing both in the geographical spread of the disease and its intensity. Unfortunately, the trend is towards the increase in *P. falciparum* malaria. This is very pronounced in the urban areas and also in areas with irrigation. The problem of malaria is further complicated by the migration and as a result its uncontrolled spread into new areas. In this context our capacity to fight malaria is diminishing because of the technical, financial and operational problems. It is therefore, important that research is intensified in areas that can remove technical obstacles and also make the field operations cost-effective and sustainable.

During the past one year Malaria Research Centre has expanded the research activities to new areas of basic and applied research. In particular, the work on sibling species complex has been extended to cover *Anopheles fluviatilis* resulting in the discovery of the sibling species complex. The work on biochemical genetics has been extended to study the mode of inheritance of the markers and the genes for resistance. Field studies have been taken up to investigate the behaviour of *Anopheles culicifacies* complex supported by the laboratory investigations.

Important vectors such as the *An. minimus* and *An. sundaicus* have been colonized. Biology of these vectors have been investigated in nature and genetic studies are in progress. These basic researches in vector biology have resulted in better understanding of the biology of vectors, eventually leading to the development of more scientific control of vectors. Ecological studies in the endemic areas have shown that the vector behaviour varies from one area to another and also seasonally. Therefore, malaria control operations should be designed to account for the vector biology before large-scale operations are planned.

An important area of vector control that has been investigated during the year is the role of neem and its products in malaria control. The repellent action of neem oil has been investigated and found to be superior to other methods of personal protection, and it is currently under consideration for introduction into the programme. A similar progress has been made in regard to the insecticide impreg-

nated bednets and we are hoping a major change in malaria control strategy would be the introduction of insecticide treated bed nets in the control of malaria, particularly in areas where the vectors are refractory to residual spray. It has already been demonstrated that the use of bednets is cost-effective and sustainable. In some areas bed nets are more effective in malaria suppression than the residual spray.

The centre is collaborating with CDRI, Lucknow on drug trials, and two drugs one on the anti-relapse and the other Chinese drug Artimisinin have undergone clinical trials. Epidemiological investigations have been carried out at various places in the country and the information elicited has been used in stratification, developing control strategies and in the modelling.

Bioenvironmental control programme is under introduction in Karnataka and already it has been demonstrated that the method is feasible, cost-effective and superior to the spraying of insecticides. We are hoping that in the coming few years most areas under spray will come under this strategy developed by the MRC.

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HIGHLIGHTS

1. *An minimus* population from Assam were identified as species A with the help of diagnostic enzyme octanol dehydrogenase.

2. Study on the bionomics of *An. fluviatilis* sibling species in districts Hardwar, Nainital and Sundergarh confirmed previous year's observations that i) species S predominant in Sundergarh was highly anthropophagic, and ii) species T and U found sympatric in Hardwar and Nainital were almost zoophagic. This difference in the host preference of these sibling species seems to have an important bearing on the malaria incidence in the areas of their distribution.

3. Age-specific insemination rates among the members of *An. culicifacies* complex was studied at a sex ratio of 1:1. Species C had the highest rate of insemination (60%) by 5th day, and the shortest median insemination rate. The maximum insemination rate of 40% was observed in species A and B. The insemination rate of species A under natural conditions in field was 92%.

4. Average survival rate for *An. culicifacies* species A was estimated in the field from nulliparous and parous data. Duration of gonotrophic cycles I and II were calculated to be 4 and 2 days respectively, and the probability of survival through sporogonic cycle was 0.195. Species A mosquitoes are capable of transmitting *P. vivax* for 3.8 days and *P.falciparum* for 3.6 days after the completion of sporogonic cycle.

5. Parasite bank now has 95 isolates of *P. falciparum* adapted to *in-vitro* culture. These isolates are from different parts of the country and most of these have been characterized for drug sensitivity and enzyme variations.

6. *P. vivax* exo-erythrocytic schizonts were developed in hepatoma cell line (Hep G_2 A 16) infected with sporozoites produced in *An. stephensi*. This is the first time *P. vivax* exo-erythrocytic schizonts were produced in India. Now that the procedures are standardized hepatic cells can be infected with sporozoites to get a higher yield of schizonts.

7. Complete cycle of *P. yoelii yoelii* and *P. yoelii nigeriensis* has been established and is being maintained for various studies.

8. *P. falciparum* isolates have been cloned using limiting dilution method. Clones obtained from each isolate have been characterized for growth, seropositivity against specific antibodies, drug sensitivity and isoenzyme variation. Clones resistant and susceptible to chloroquine have been isolated from single isolates and are being maintained.

9. Clones producing antibodies against *P. vivax* circumsporozoite protein have been isolated. Studies are in progress to assess the specificity and sensitivity of the antibodies.

10. Analysis of *P. vivax* isolates for enzyme electrophoretic variations revealed that all isolates are not of single clonal origin. Isolates with single clones were 85% and with multiclones 15%. No correlation was observed between clonal composition and the intensity of malaria transmission.

11. *P. falciparum* MSA 2 gene was amplified by PCR using 23 mer primers. Amplified products were cloned and sequenced. Comparison with the published sequences of MSA 2 revealed conformity as well as some distinctive features in the MSA 2 product amplified in this study.

12. Synthetic peptide RESA was found to be more accurate in estimating malaria endemicity than *P. falciparum* crude antigen.

13. In studies initiated to understand the immune responses during infection with chloroquine resistant and susceptible *P. berghei* infected mice, cellular and humoral responses were lower in chloroquine resistant infected mice than in those with susceptible parasites. And also gametocyte conversion rate was more in susceptible parasites.

14. A pilot study to find out the feasibility of studying terrain-based probability of mosquitogenic conditions using Geographic Information System (GIS) was initiated jointly with IIRS, Department of Space, Dehradun. Preliminary results revealed that by digital terrain modelling, the possible water logging zones can be predicted.

15. A study to map water bodies by remote-sensing revealed both positive and negative correlation with different water bodies. The technique was sensitive to map qualitative and quantitative changes in large water bodies and thus *C. quinquefasciatus* breeding potential could be estimated. As minor water bodies can not be covered with the present resolution of the satellite sensors, breeding potential of

An. culicifacies, which breeds in smaller water bodies, could not be estimated.

16. A study has been initiated to study the impact of Gurgaon water supply channel on malaria transmission. Construction is in progress and water is expected to be released soon. Six villages situated on the banks have been selected for entomological and parasitological surveys.

17. An epidemiological study has been initiated to analyze urban malaria in the country. Gujarat has been taken as a first case study. Malaria cases per square kilometer have been plotted from 1978 to 1991. Out of 17 urban districts, eight districts showed increasing trend during 1978-84 while increase in 11 districts was seen during 1985-1991. Ecological parameter in these districts were analyzed to identify the relationship with malaria incidence.

18. A total of 2493 patients reported in MRC clinic between July 1988 to December 1993 and were analyzed for relapse pattern. A 29.04% relapse rate (724 patients) was observed. The number of relapses per patient varied and maximum observed were 13. 61% of the patients relapsed once.

19. Among five biolarvicides (Bti formulations) tested in the laboratory and field conditions, Bactoculicide was found most effective against larvae of different mosquito speciesincluding *An. culicifacies* and *An. stephensi*, the major malaria vectors in rural and urban areas respectively in India. The activity of 'Spherix' (*B. sphaericus* B-101) was adversely affected by exposure to sunlight in addition to other environmental factors viz. low temperature and pH for 10 h or more.

20. 'Bactoculicide' and 'Spherix' were toxic to larvae of *P. argentipes* and *P. papatasi* under experimental conditions in laboratory. There was inverse instar-susceptibility relationship.

21. Neem oil extracted from seeds of *Azadirachta indica* is an effective repellent against the mosquitoes and sandflies. 1% neem oil in kerosene burnt in lamps with a cotton wick gave 100% protection from the bites of five *Anopheles* and substantial reduction from biting nuisance of *Culex*. Similarly, 2% neem oil in mustard or coconut oil on application on the exposed body parts of volunteers gave 100% protection for 12 hours from *Anopheles* mosquito bites.

A comparative study carried out to examine the effect of neem oil and mats containing allethrin revealed that neem oil mats provided 83% reduction in biting, while with allethrin mats it was 74%.

22. Among six neem (*Azadirachta indica)* products tested as mosquito larvicide, Neemark was found most effective against larvae of *An. stephensi* (malaria vector), while Neemrich – II was most effective against larvae of *Culex quinquefasciatus* and *Aedes aegypti*. In general, of the three mosquito spp. tested *An. stephensi* was most susceptible followed by *Cx. quinquefasciatus* and then *Ae. aegypti*.

23. Field evaluation of neem oil against *Phlebotomus papatasi* in endemic area of oriental sore in Rajasthan revealed that topical application of 2% neem oil provided 97.6% protection from bites for 10 h, while 5% neem mats when used on electric heating device (trade name Good Night) provided 100% protection. Electric table fan was required to dispose vapour from the room.

ENTOMOLOGY

Studies on the *Anopheles culicifacies* Complex

Mapping the Distribution of Sibling Species

In continuation of mapping the geographic distribution of *An. culicifacies* sibling species in different parts of the country, samples from villages in Districts Waynad (Kerala) and Purulia (West Bengal) were also examined for sibling species composition. Species A and B were found sympatric in District Waynad and out of 25 ovaries examined only 3 could be identified. Of these, 2 were species A and 1 was species B. A collaborative study has been initiated with the Calcutta School of Tropical Medicine to examine the sibling species composition of *An. culicifacies* and to establish the role of sibling species in malaria transmission in Purulia district, West Bengal. Out of 69 ovaries sent for sibling species identification only 32 could be identified and all were found species B.

Bionomics and Sibling Species Composition

District Kheda (Gujarat): *An. culicifacies* were collected from a hamlet, Galteshwar of village Sarnal in Thara taluka in District Kheda. The village is situated on the banks of river Mahi and reports high malaria incidence with slide positivity rate (SPR) ranging from 30 to 50%. Analysis of sibling species composition revealed a high proportion of species A which constituted 22.2% of the total identified. Species B was the predominant (74.1%) and species C was only 3.7% (Table 1). This is the first time species A was found in Kheda district as in all earlier samples examined from Nadiad taluka only species B and C were observed.

District Alwar (Rajasthan): A survey was carried out in District Alwar in September 1993 in relation to the study on cuticular hydrocarbons profile in *An. culicifacies* sibling species. Ovaries from half gravid females were processed for sibling species identification and the rest of the body parts from these mosquitoes are being analyzed for cuticular hydrocarbons in collaboration with Defence Research Development Establishment Laboratory, Gwalior.

Regarding sibling species composition in the study villages, species A,B and C were found sympatric. Species A was predominant comprising 67.5% of the total identified and was polymorphic for i[1]

Table 1. *An. culicifacies* sibling species composition in the districts surveyed

Districts ident.	Total	Species A*				Species B	Species C	B/C
		A	A/A'	A'	Total			
Kheda	54	4	5	3	12	40	2	0
Alwar	213	28	69	34	131	77	5	0
Allahabad	205	6	67	83	156	41	8	0
Sundergarh	708	1	0	1	2	184	516	6

*In all the areas i^1 inversion was found to be in balanced morphic conditions; A – $+^{i1}/+^{i1}$ A/A^1 – $+^{i1}/i^1$, A' – i^1/i^1

inversion. Proportion of species B was 36.15% and species C was found in very low numbers constituting 2.35% of the total identified (Table 1).

Blood meal analysis of the above sample revealed that none of the species B and C was fed on human blood, whereas, 3 out 132 species A were found positive for human blood with HBI of 0.02.

District Allahabad (U.P.): Ovaries from half gravid females of *An. culicifacies* collected during June, July and August 1992 from villages Bemera, Hinauti, Bashera, Kalyan Pur etc. which fall in canal irrigated and stone quarry areas of District Allahabad were examined for sibling species composition. Analysis of the data revealed high proportion of species A in these villages. Species A was found polymorphic for i^1 inversion and comprised 76% of the total identified. Species B and C constituted 20% and 3.9% respectively of the total identified (Table 1). The cytologically identified specimens were subjected to blood meal analysis to study the host preference. All specimens of species B and C were found fed on bovine or other animals blood whereas a good proportion of species A was found fed on human blood with HBI of 0.096. High malaria incidence in these villages may be attributed to the predominance of species A, an established vector of malaria, which was also found to be anthropophagic.

District Sundergarh (Orissa): In District Sundergarh a study was initiated to examine *An. culicifacies* sibling species composition and their seasonal prevalence. Villages representing different ecosystems were selected and monthly adult collections were made in the year 1992

and 1993. Ovarian polytene chromosomes of half gravid females from these collections were examined for sibling species identification. The data revealed that species B and C were sympatric in all the study villages and species A was found occassionally in very low numbers. Species C was predominant comprising 72.9% of the total identified and species B was approximately 26% and species A was 0.28% (Table 1).

In riverine villages (Jamsera and Jabaghat) the relative proportion of species B and C did not change in pre-monsoon (January - June) and post-monsoon (July - December) months. Species B was 22.0% and species C 77.7% in pre-monsoon collections and their percentages were 23.5% and 76.5% respectively in post-monsoon collections. Whereas, in villages, Manko and Birkera, in hilly forest areas an appreciable increase in species B proportion was observed in post-monsoon months. The proportion of species B increased from 22.0 to 41.5% in post-monsoon period and species C were 78 and 58.5% in pre- and post-monsoon months respectively. Whether this increase in species B was due to availability of preferred breeding sites or favourable climatological conditions needs to be explored.

Results of the blood meal analysis revealed that both species B and C were primarily zoophagic in the study villages. However,

Loading of samples on gel for electrophoresis

a small proportion of both the species was found fed on human blood and the HBI of species C (0.025) was higher than that of species B (0.01).

Calculation of Survival Rates from Field collected Species A of *An. culicifacies* Complex using Parous Rates

The average survival and biting rates may be estimated in the field under conditions of variable recruitment rate from relatively short runs of nulliparous and parous data. All anopheline mosquito collections were carried out for 27 consecutive days both in indoors on resting mosquito population and outdoors on tethered buffaloes (that were used as animal baits) of biting mosquito population. Indoor collections were made by two insect collectors in three empty cattlesheds for a period of 15 minutes between 0100 and 0715 hours. Bait collections were performed by two insect collectors on four tethered cattle for a period of 15 minutes at the beginning of each hour between 1800 and 0600 hours. All the anopheline mosquitoes were caught while in the process of landing, probing or feeding on the bait. All the anopheline species thus collected were separated and only *An. culicifacies* were used for further analysis. *An. culicifacies* s.l. thus

Table 2. Estimation of the survival rate and duration of gonotrophic cycles I and II of field collected *An. culicifacies* species A mosquitoes in village Dhera, U.P., during April 1993

Sampling method	Bait coll. (outdoor)	Hand catch (indoor coll.)
Sampling time	1800–0600 h	0700–0715 h
Temperature (C°)	23.5 + 2.7	25 + 1.02
RH (%)	67.1 + 8.3	69 + 4.6
No. of collections	27	27
Total mosquitoes	1097	2387
Man hour density	20.3	58.94
Pre–gravid rate (%)	77.2	65.6
Parous rate	48.4	59.8
Survival rate/G. cycle – 1	0.548 + 0.02	0.704 + 0.01
Oviposition cycle I	4	4
Oviposition cycle II	2	2

collected in indoor as well as outdoor collections were separated according to their abdominal conditions as unfed, fullyfed, half gravid and gravid mosquitoes. The ovaries of unfed and fully fed mosquitoes were dissected in normal saline (0.67%). The tracheolar skins on the ovary was observed under compound microscope for parous condition (Detinova, 1962). The ovaries of half gravid mosquitoes were removed and stored in modified Carnoy's fixative. *An. culicifacies* population at Dehra during this study period comprised 98% species A and 2% species B.

The modified formula of Mac Donald (1957) used by M.H. Birley and Rajagopalan (1981) and Service (1994) was used to estimate the survival rate and the duration of first and second gonotrophic cycles (Table 2).

The daily survival rate was calculated using the formula

$$p = \sqrt[g]{\text{Parous rate}}$$

where 'p' is the daily survival rate, 'g' is the duration of gonotrophic cycles and the parous rate of the pre-gravid mosquitoes was obtained by the degree of coiling of tracheolar skins on ovaries (Detinova, 1962) (Fig 1).

Using the period of extrinsic cycle of 9 and 10 days for *P. vivax* and *P. falciparum* respectively (Bruce-chwatt, 1985), the probability of survival of the mosquito through sporogonic cycle was calculated using the formula

$$\text{Prob. Surv. Sporo. Cycle} = p^n$$

where 'p' is the daily survival rate and 'n' is the duration of sporogonic cycle in mosquito.

The expectation of infective life (EIL) or longevity factor (v) for species A was calculated, using 'p' (the daily survival rate) and 'u' (the duration of gonotrophic cycle) following the methodology given by Birley, 1984.

$$v = p^{n/u} / 1\text{-}p$$

A total of 2387 and 1097 mosquitoes were collected from indoor resting and outdoor animal biting population of *An. culicifacies* in a 27 consecutive collections. The average per man

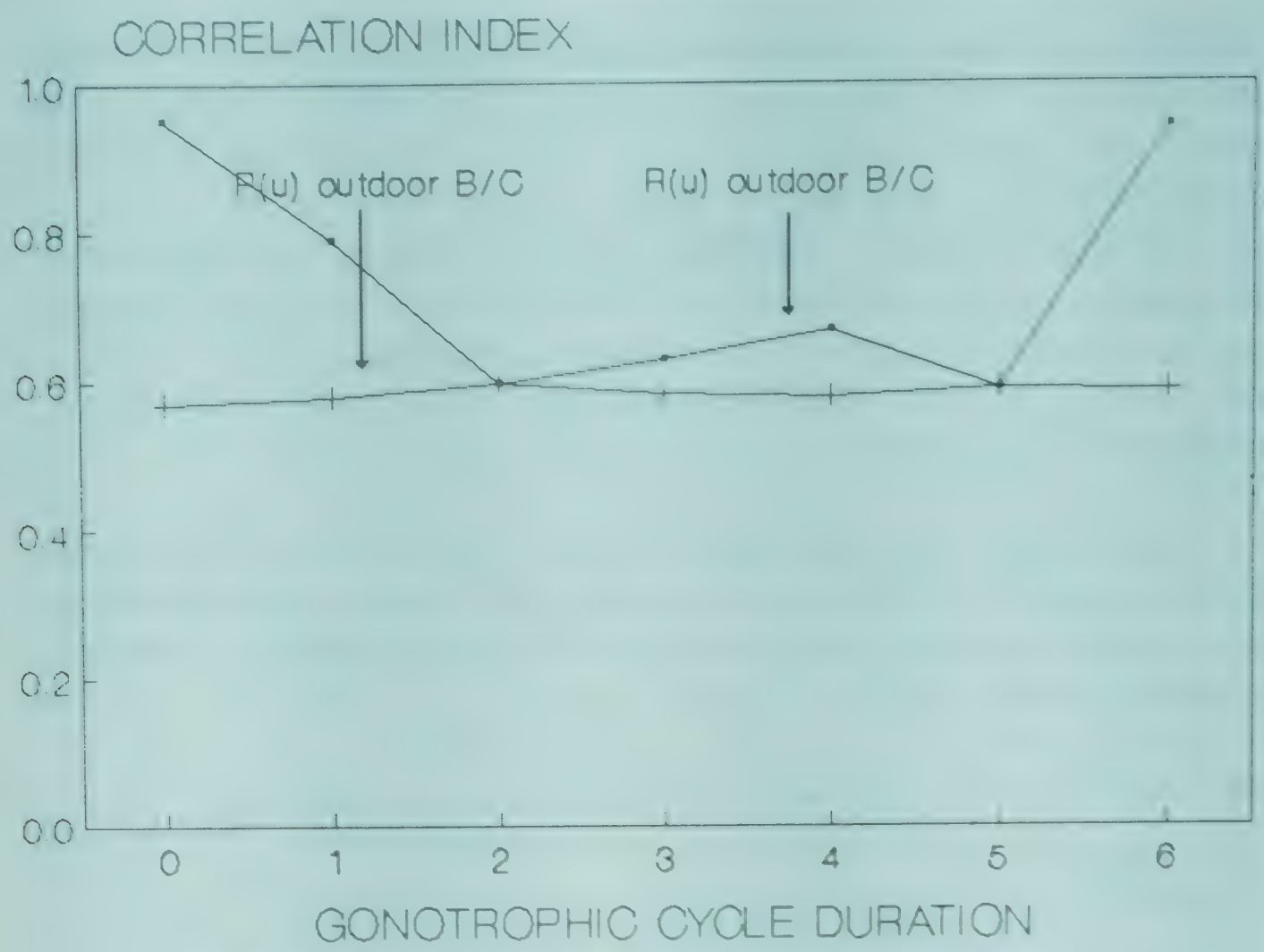


Fig. 1: Estimation of duration of gonotrophic cycles I and II and the survival rate per gonotrophic cycle 1

hour density was 59 in indoor collections and 20 in biting collections. In the indoor resting populations the proportion of pregravid mosquitoes was significantly lower than that of bait collections (χ^2 = 47.5, $p<0.01$) while the parous rate of resting mosquitoes was significantly higher in the indoor resting mosquitoes (χ^2 = 18.0, $p<0.025$). As lower pre-gravid rate and higher parous rate in the indoor resting populations would lead to over estimation of the survival rate of the mosquitoes, so only the bait collections were taken into account to estimate the survival rate and the duration of gonotrophic cycles. The duration of gonotrophic cycles I and II were estimated to be of 4 and 2 days respectively in both field and laboratory conditions for species A. The daily survival rate and the survival rate per gonotrophic cycle I (taking the duration of gonotrophic cycle I as 4 days) for resting, biting and the laboratory line of species A was 0.916, 0.834 and 0.776 for daily survival rate and 0.704, 0.548 and 0.87 for survival rate per gonotrophic cycle respectively. Field collected species A was compared with values estimated for laboratory reared species A population. The survival rate per gonotrophic cycle I in biting population collected in field was found to be significantly lower than the value calculated for colonized mosquitoes

which could be due to lower mortality during oviposition while the daily survival rate was found to be similar. The probability of survival of species A of *An. culicifacies* and their expectation of infective life through sporogonic cycle for *P. vivax* and *P. falciparum* with extrinsic periods of 9 and 10 days respectively were found to be 0.454, 0.195 and 0.102 for *P. vivax* and 0.415, 0.163 and 0.08 for *P. falciparum* in indoor resting, outdoor biting and laboratory lines of species A respectively. Field collected biting population showed significantly higher survival through sporogonic cycle than laboratory line ($F = 10.50$; $p < 0.01$). The expected life of infectivity (EIL) was 9.77, 4.0 and 2.52 days for *P. vivax* and 9.56, 3.83 and 2.37 days for *P. falciparum* respectively for field collected indoor resting, outdoor biting and laboratory adapted species A. No significant difference (Fig. 1) was found between biting and laboratory population (Table 3).

Insemination Rates Among the Sibling Species

Twelve replicate cages, each containing laboratory lines of 50 freshly emerged male and female mosquitoes of *An. culicifacies* in 1:1 ratio for each of the sibling species A, B and C were held in an insectry, maintained at 28±2°C temperature, 75±5% relative humidity

Table 3. Survival and transmission potential of *An. culicifacies* species A under field and laboratory conditions

Parameter	Indoor coll. (R/C)*	Outdoor coll. (B/C)**	Species A (lab) estimated
Daily S.R (p)	0.916	0.834	0.776
S.R./G.C – I	0.704	0.548	0.87
Prob. SPR Cycle			
P. vivax	0.454	0.195	0.102
P. falciparum	0.415	0.163	0.08
EIL			
P. vivax	9.77	4.0	2.52
P. falciparum	9.56	3.83	2.37

*– Indoor resting populations; ** – Biting populations of mosquitoes; EIL – Expected period of infective life in the mosquito (in days) = $p^{n/u}/1-P$ (Birley, 1984); Prob. of surv. thro. sporo. cyc. – pn; p = daily S.R.; n = duration extrn. cyl. *Pv* = 9, *Pf* = 10 days

and with a diurnal rhythm of 16 hours light and 8 hours dark having a crepuscular period of 90 minutes. Soaked raisins and cotton pads containing glucose solution were provided to the mosquitoes as a source of nutrition.

From day 1(approximately 24 hours after emergence), all the female mosquitoes present in two cages for each sibling species were dissected for spermathecae and were examined under compound microscope for the presence of sperms in them. By day 6 all the female mosquitoes present in all the twelve cages for each sibling species were examined for insemination and the percentage of mosquitoes that were inseminated was calculated (Tables 4-5).

Table 4. Age–specific insemination rates of *An. culicifacies* sibling species

Age in days	Number of mosquitoes					
	Species A		Species B		Species C	
	Dissec.	+ve	Dissec.	+ve	Dissec.	+ve
1	50	3	49	9	48	5
2	48	12	50	15	50	12
3	45	17	46	17	48	14
4	49	25	44	25	49	18
5	42	23	46	33	44	20
6	44	31	41	35	46	27

Dissec. – Number of mosquitoes dissected; +ve – Number of mosquitoes whose spermathecae were found positive for sperms

Table 5. Regression analysis of insemination rates of sibling species A,B and C of *An. culicifacies* with the sex-ratio of 1:1 under laboratory conditions

Sibling species	Reg. equation	Reg. coeff. (r)	$I_{(50)}$ (days)	$I_{(99.9)}$ (days)
A	$Y = -1.94 + 7.03\ x$	0.93	7.39	14.5
B	$Y = 9.84 + 4.83\ x$	0.84	8.3	18.7
C	$Y = 4.0 + 9.68\ x$	0.95	4.7	9.9

The age specific variation in the insemination rates among the members of *An. culicifacies* complex was analyzed using regression analysis (Sokal and Rohlf, 1969). When the population has males and females in 1:1 ratio species C showed the highest insemination rate (60%) by day 5 after emergence. In species A and B a maximum of 37.1% would be achieved by day 6. The median insemination rate of species C was the shortest 4.7 days. These values are far less than the values obtained in field (92% inseminated) (Fig. 2).

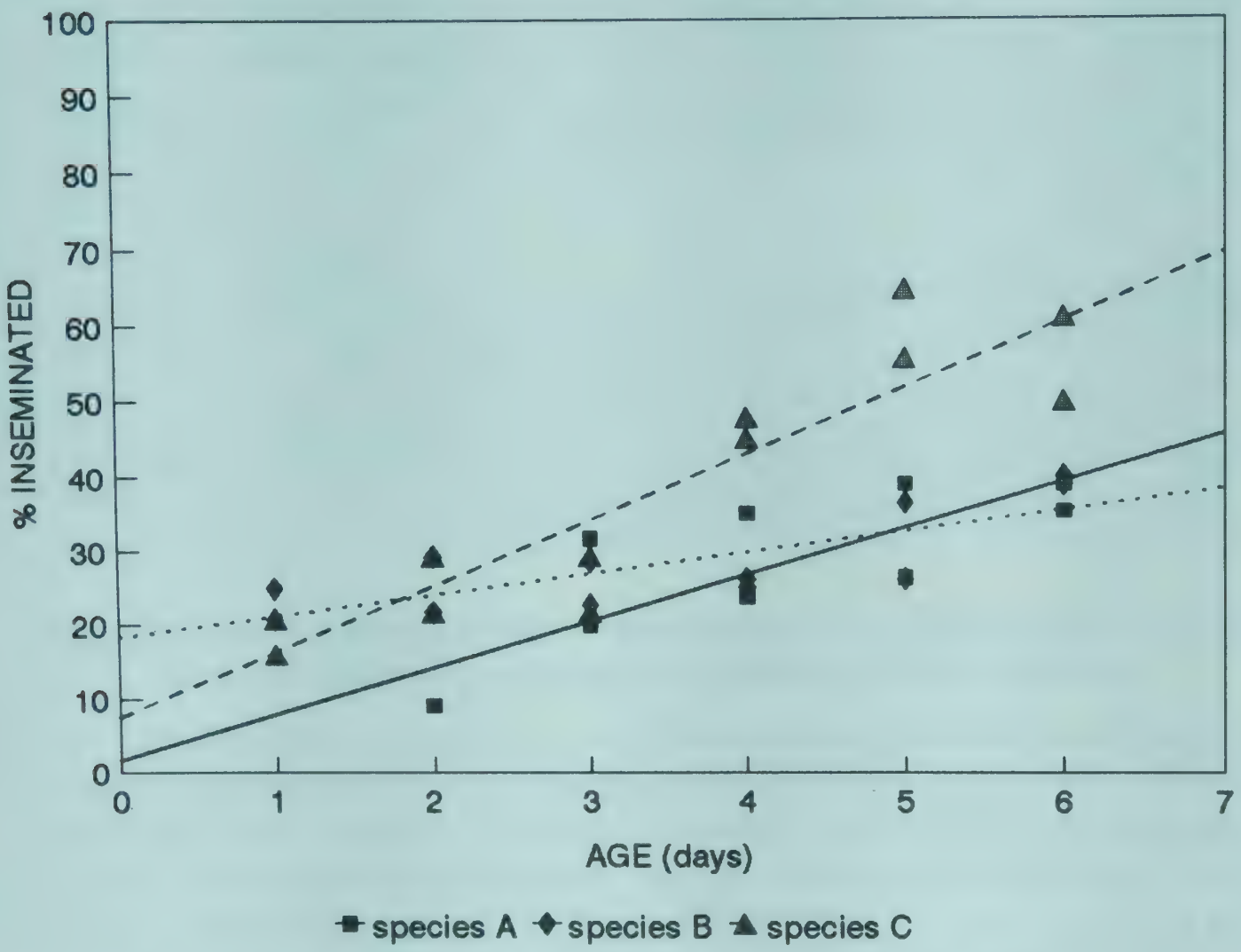


Fig. 2: Regression equations showing the effect of age on the insemination rate of *An. culicifacies* sibling species

Insecticide-resistance in species A and B in District Sonepat, Haryana

A study to monitor responses of *An. culicifacies s.l.* and sibling species to malathion in District Sonepat, Haryana, where malathion was introduced in public health programme in 1982 to control an epidemic of malaria. As given in Table 6, in February 1993, the proportion of species A was 70%, while in July it decreased to 25%. The proportion of species B in post-monsoon months (after July) in 1993, was in the range of 75-96.

Table 6. *An. culicifacies s.l.* densities, species composition and susceptibility to different insecticides in District Sonepat, Haryana

Period (month) 1993	MHD	Species composition		% mortality		
		% Sp. A ( )	% Sp. B ( )	DDT (No. exp.)	Dieldrin (No. exp.)	Malathion (No. exp.)
Jan	9.4	–	–	–	–	–
Feb	7.0	70 (12)	30 (5)	–	–	–
Jul	19.2	25 (5)	75 (15)	66 (80)	0 (60)	57 (224)
Aug	5.9	20 (1)	80 (4)	–	–	30 (30)
Sept	1.5	–	–	–	–	–
Oct	9.4	–	–	–	28 (39)	31 (58)
Nov	17.0	25 (15)	75 (45)	–	–	29 (390)
Dec	19.5	4 (1)	96 (25)	22 (55)	13.3 (45)	36 (50)

MHD – Man hour density; (No. exp.) – No. of mosquitoes exposed; ( ) – No. of mosquitoes identified for sibling species

Table 7. Susceptibility of *An. culicifacies* species A and B to 5.0% malathion and 4.0% DDT in bioassay tests in District Sonepat, Haryana

Period (month) 1993	% resistance* (No. Exp.) *An. culicifacies* s.l.	Species composition		% resistance	
		% Sp. A (n)	% Sp. B (n)	Sp. A (n)	Sp. B (n)
				5% malathion	
Nov.	73 (99)	25 (15)	75 (45)	50 (8)	61 (36)
				4% DDT	
Dec.	10 (34)	4 (1)	96 (25)	100 (4)	94 (17)

*Mosquitoes were scored for mortality and processed for sibling species immediately after exposure for 1 h; (No. exp.) – Number of mosquitoes exposed; (n) – Number of ovaries identified

The per cent mortality of *An. culicifacies s.l.* in insecticide bioassays with DDT in July was 66 while in December it decreased to

22. In bioassays with dieldrin the per cent mortality ranged between 0 and 28 and to malathion it ranged between 29-57%, the population was found to be susceptible to fenitrothion, propoxur and deltamethrin. The above toxicological bioassays on *An. culicifacies* have indicated the population to be triple-resistant i.e. to DDT, dieldrin and malathion.

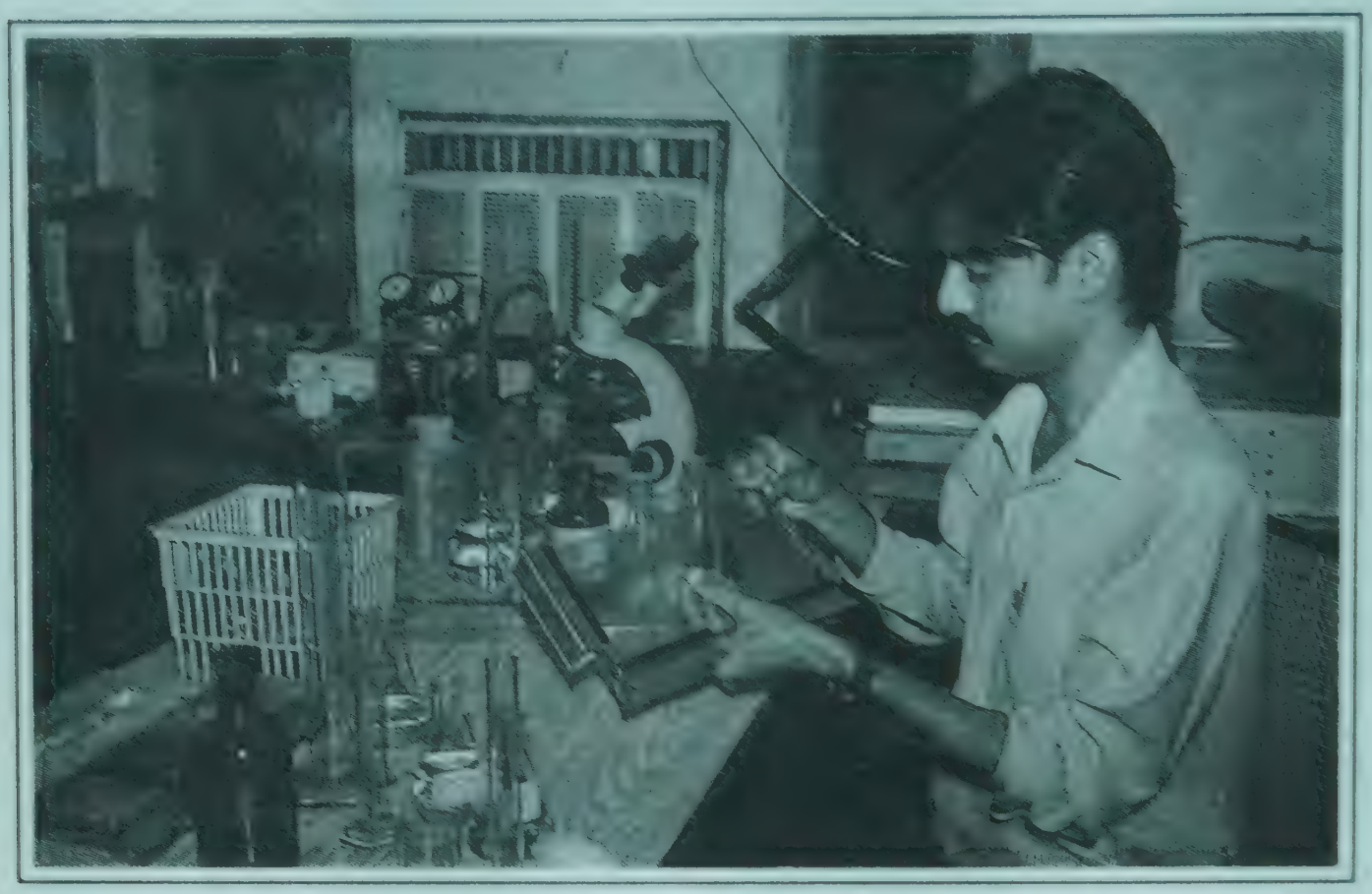
In studies to determine the responses among the members of sibling species (Table 7), species A registered 50% resistance and species B 61% against malathion and against DDT species A was completely resistant whereas species B was 94% resistant (Table 7). Compared to the observed resistance in species A in 1992 (MRC Annual Report, 1992) in 1993 a three times increase in malathion-resistance was noticed (Table 6).

Time-mortality response studies in *An. culicifacies s.l.* with malathion in 1993 have also indicated a substantial increase in the computed lethal time (LT_{50}) over the observed value of 73.8 min in August 1992 thereby indicating higher levels of resistance in the population. In July 1993, the computed LT_{50} value was 65.5 min which increased to 116.7 min in November 1993 and further to 140.5 min in December 1993.

Studies on Isoenzymes

In our earlier studies on enzyme electrophoresis for identification of sibling species complexes of *An. culicifacies* some ten enzyme systems were screened. Lactate dehydrogenase (LDH) enzyme was found to be of diagnostic value. But the limitation of this enzyme is that it cannot distinguish between sibling species A and D and B and C. A study was therefore, initiated to screen some more enzymes to distinguish species B from C and species A from D in areas where these species are sympatric.

An. culicifacies, collected from some villages of Nadiad, District Kheda (Gujarat) and Rourkela (Orissa) were screened. These two areas were selected on the basis of cytogenetical identifications which revealed that sibling species B and C are sympatric with the predominance of species B. Results of the study are tabulated (Tables 8-11). *An. culicifacies* from Nadiad were screened for Aspartate aminotransferase (AAT), Adenylate kinase (AK), Xanthine dehydrogenase (XDH), Hydroxyacid dehydrogenase (HAD), Hexokinase (HK), Fumerase (FUM), Malate dehydrogenase (MDH),Octanol dehydrogenase (ODH) and lactate dehydrogenase (LDH). In Nadiad population, out of these eight enzyme systems studied, AAT, HAD, AK, XDH, MDH, ODH



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were found to be polymorphic whereas, HK and LDH were monomorphic. In the field population from Rourkela, AAT, HAD, XDH and AK were examined. All the enzymes except AK were polymor-

Table 8. Enzyme phenotypes observed in *An. culicifacies s.l.* from Nadiad District Kheda, Gujarat

Enzymes	Total sample	Enzyme phenotype		
		FF	SF	SS
Aspartate aminotransferase (AAT)	84	66	18	–
Hydroxyacid dehydrogenase (HAD)	50	50	–	–
Adenylate kinase (AK)	63	61	1	1
Hexokinase (HK)	47	47	–	–
Xanthine dehydrogenase (XDH)	28	27	–	1
Malate dehydrogenase (MDH–2)	79	74	4	1
Octanol dehydrogenase (ODH)	32	30	–	2
Lactate dehydrogenase (LDH)	1	–	–	1

Table 9. Enzyme phenotypes observed in species B* from Nadiad, District Kheda, Gujarat

Enzymes	Total samples	Enzyme phenotype		
		FF	SF	SS
Aspartate aminotransferase (AAT)	36	27	8	1
Hydroxyacid dehydrogenase (HAD)	30	25	4	1
Adenylate kinase (AK)	38	34	2	2
Hexokinase (HK)	31	31	–	–
Xanthine dehydrogenase (XDH)	15	10	–	5
Malate dehydrogenase (MDH–2)	41	34	4	3
Octanol dehydrogenase (ODH)	28	27	1	–
Lactate dehydrogenase (LDH)	7	–	–	7

*Cytologically identified species

Table 10. Enzyme phenotypes in *An. culicifacies s.l.* from District Sundergarh, Orissa

Enzyme	Total samples	Enzyme phenotype		
		FF	SF	SS
Aspartate aminotransferase (AAT)	135	112	19	4
Hydroxyacid dehydrogenase (HAD)	162	158	3	1
Adenylate kinase (AK)	20	20	–	–
Xanthine dehydrogenase (XDH)	41	21	2	12

phic. All enzymes were autosomal in nature. AAT, HAD, AK and LDH were found to be monomers and MDH-2 dimer. None of these enzymes except HK and ODH which are yet to be confirmed were found to be diagnostic in distinguishing between different sibling species. More enzymes are being screened to find diagnostic enzymes, if any.

Resistance to Insecticides in *An. subpictus* in District Sonepat, Haryana: In 1989-1990, *An. subpictus* registered 63% mortality in malathion bioassays. In 1993, during August to October, *An. subpictus*

Table 11. Enzyme phenotypes observed in *An. culicifacies* sibling species B* and C* from District Sundergarh, Orissa

Enzyme	Total samples	Enzyme phenotype					
		Sibling species					
		B			C		
		FF	SF	SS	FF	SF	SS
Aspartate aminotransferase (AAT)	20	11	1	–	5	1	1
Hydroxyacid dehydrogenase (HAD)	22	15	–	–	6	–	1
Adenylate kinase (AK)	16	12	–	–	3	–	–
Xanthine dehydrogenase (XDH)	14	10	–	–	2	1	–

*Cytologically identified species

were available in high abundance and were subjected to toxicological bioassays. The per cent mortality registered in bioassays against DDT was 11.25, dieldrin 6.6, malathion 84 and fenitrothion 100 respectively. In time-mortality response studies, the computed LT_{50} values against malathion and fenitrothion was 39.12 min and 21 min respectively.

Studies on the *Anopheles fluviatilis* Complex

Mapping the Distribution of Sibling Species

Spot surveys were carried out in Districts Allahabad (Uttar Pradesh), Kheda (Gujarat) and Kamrup (Assam) to examine the sibling species composition (Table 12). Only species T was found in Districts

Table 12. *An. fluviatilis* sibling species composition in the districts surveyed

District (State)	Total collection	Total identified	Species		
			S	T	U
Allahabad (U.P.)	51	36	0	36	0
Kheda (Gujarat)	36	29	0	29	0
Kamrup (Assam)	55	25	0	0	25

Allahabad and Kheda. All the 36 and 29 samples that could be identified from Districts Allahabad and Kheda respectively were species T and no specimen of species S and U was found. Whereas, in District Kamrup all 25 specimen identified were of species U.

Bionomics of Sibling Species in Districts Nainital (U.P.) and Sundergarh (Orissa)

In 1991 a study was initiated on the bionomics of *An. fluviatilis* sibling species to explore their role in malaria transmission in selected villages of Districts Hardwar, Nainital and Sundergarh. The study was continued for the second successive year in Districts Nainital and Sundergarh and the results obtained confirmed the earlier observations.

In district Nainital *An. fluviatilis* population started building up from September onwards and extended up to March-April. Densities of *An. fluviatilis* sensu lato were high and majority of the populations were found resting in the cattlesheds. In villages Nai Avadi and Pachaunia selected in Bhabar area, all 52 specimen identified from September - November collections were species T, whereas in villages Tilpuri and Matkota in terai area both species T and U were found constituting 64.7% and 35.3% respectively of the total identified. No specimen of species S was found in any of the selected villages (Table 13).

In villages Manko and Birkera of District Sundergarh which are in hilly forest area, all the specimens identified from 5 collections were species S (Table 14). In 1991 collections species S constituted 97% of the total identified hence species S remains the predominant species in the selected villages of this district.

Table 15 show results of blood meal analysis reflecting the host preference of these sibling species. In District Nainital where species T and U are sympatric, species U was found exclusively zoophagic whereas 172 out of 175 species T tested were found fed on bovine blood and 3 were mixed positive both for human and bovine blood with HBI (Human Blood Index) of 0.02. In District Sundergarh where species S constitutes almost whole of *An. fluviatilis* populations more than 90% of the total tested were found fed on human blood and HBI was 0.94. These results confirm our earlier observations that species S is highly anthropophagic whereas species T and U are primarily zoophagic. This difference in the host preference

Table 13. District Naini Tal (Uttar Pradesh) – *An. fluviatilis* sibling species composition

Month/ Year	Total collected	Total identified	Species		
			S	T	U
Villages Nai Avadi, Pachaunia (Bhabar area)					
Sept 92	43	10	0	10	0
Oct 92	60	28	0	28	0
Nov 92	37	14	0	14	0
Total	140	52	0	52	0
Villages Tilpuri, Matkota.(Terai area)					
Sept 92	14	4	0	3	1
Oct 92	24	5	0	3	2
Nov 92	137	75	0	51	24
Dec 92	7	5	0	5	0
Feb 93	48	44	0	25	19
Mar 93	19	17	0	14	3
Apr 93	42	40	0	22	18
Total	291	190	0	123	67

Table 14. District Sundergarh (Orissa) – *An. fluviatilis* sibling species composition

Month/ Year	Total collected	Total identified	Species		
			S	T	U
Villages Birkera, Manko (Hilly forested area)					
Jul 92	2	1	1	0	0
Aug 92	16	13	13	0	0
Sept 92	23	14	14	0	0
Oct 92	6	2	2	0	0
Nov 92	17	5	5	0	0
Total	64	35	35	0	0

Table 15. *An. fluviatilis* sibling species – Results of the blood meal analysis

Species	Total tested	Human +	Bovine +	Mix +	Non-reactive	HBI
			District Naini Tal			
T	175	0	172	3	0	0.02
U	67	0	64	0	3	0
			District Sundergarh			
S	35	33	2	0	0	0.94

of these sibling species has an important bearing on the malaria incidence in the areas of their distribution.

Ultrastructural Study of the Egg Surface Morphology of Sibling Species T and U

For this study the eggs from cytologically identified single female cultures of species T and U were air dried, coated with gold in ion-sputter coating apparatus and examined under scanning electron microscope.

Figure 3 shows lateral view of the egg of species T. The egg like that of other anopheline species is boat shaped with convex dorsal side and concave ventral side. On the ventral side the egg has fine frill with wavy margin and lobed tubercles at the anterior and posterior ends of the deck. At the anterior pole of the egg very prominent micropylar collar consisting of long recurved tubercles with spiny projections and enclosing a circular micropylar orifice is observed (Fig. 4). No differences were observed in the number of lobed tubercles at the anterior and posterior ends of the deck, the pattern of cells formed by the tubercles on the outer chorionic membrane and the micropylar region between the eggs of species T and U. The number of ridges on the egg float varied from 17-20 in both the species.

Two types of eggs showing variation in the frill surrounding the deck have been observed in both species T and U. In type one the deck is continuous and surrounded by frill all along its margin (Fig. 5). In second type the deck is not continuous and chorion in the middle of the egg is fused on ventral side dividing the deck into two parts each surrounded by the frill in circular fashion (Fig. 6).

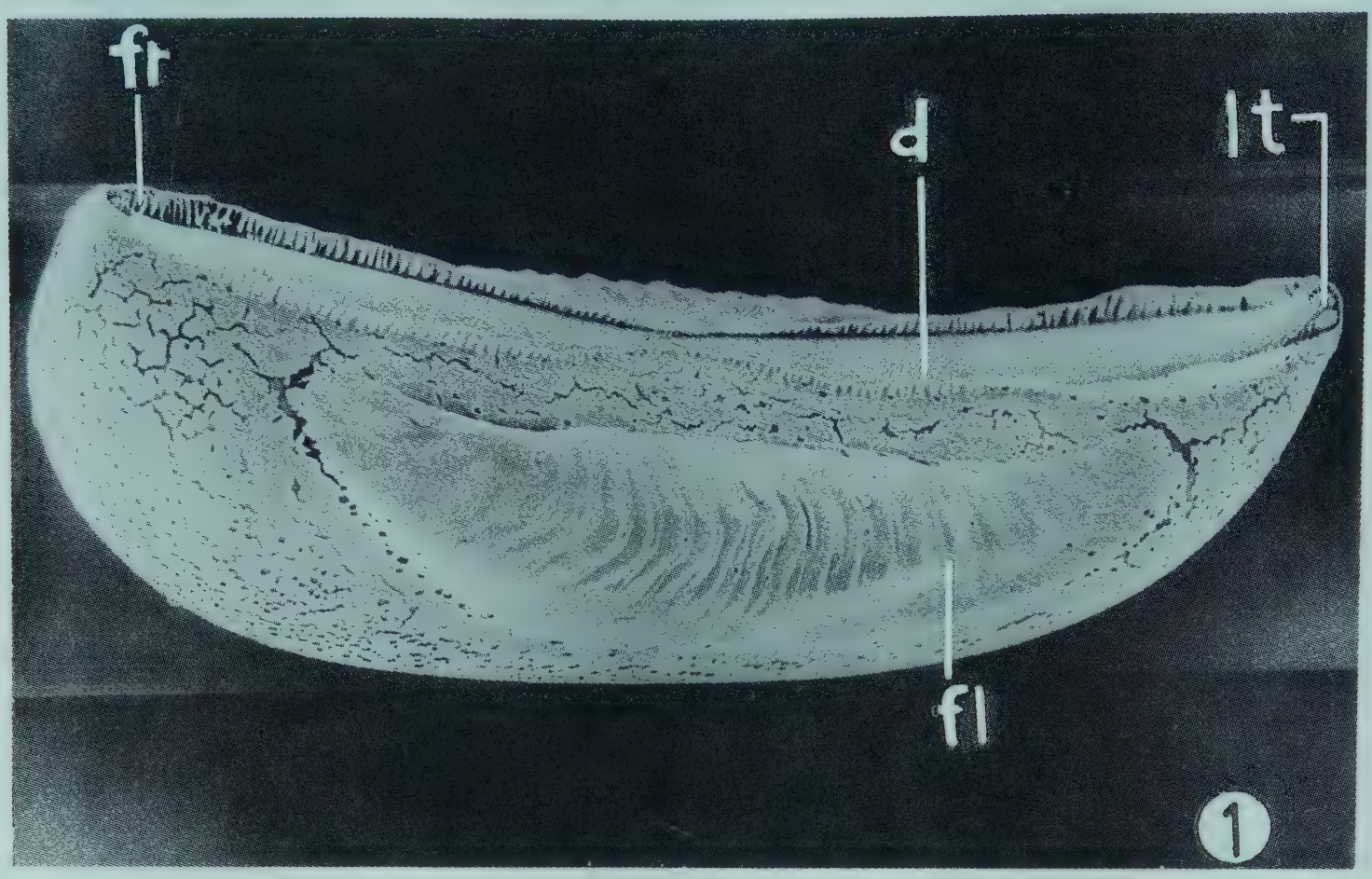


Fig.3: Scanning electron micrograph showing lateral aspects of the egg of *An. fluviatilis* species T [d = deck, fl = float, fr = frill; LT = lobed tubercle, x 500]

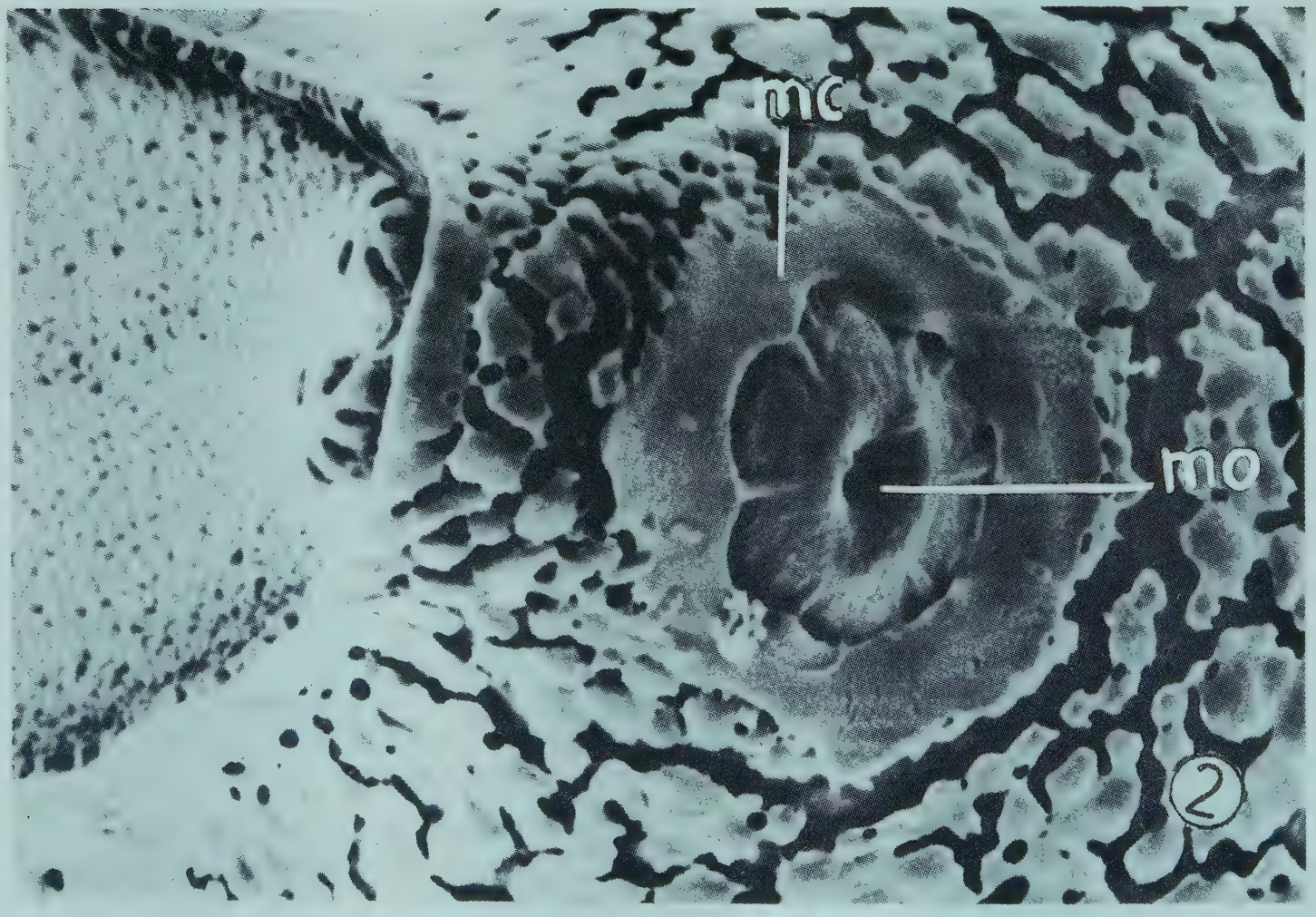


Fig. 4: Highly magnified view of anterior pole of the egg showing micropylar region
[mc = micropylar collar; mo = micropylar orifice, x 5,000]

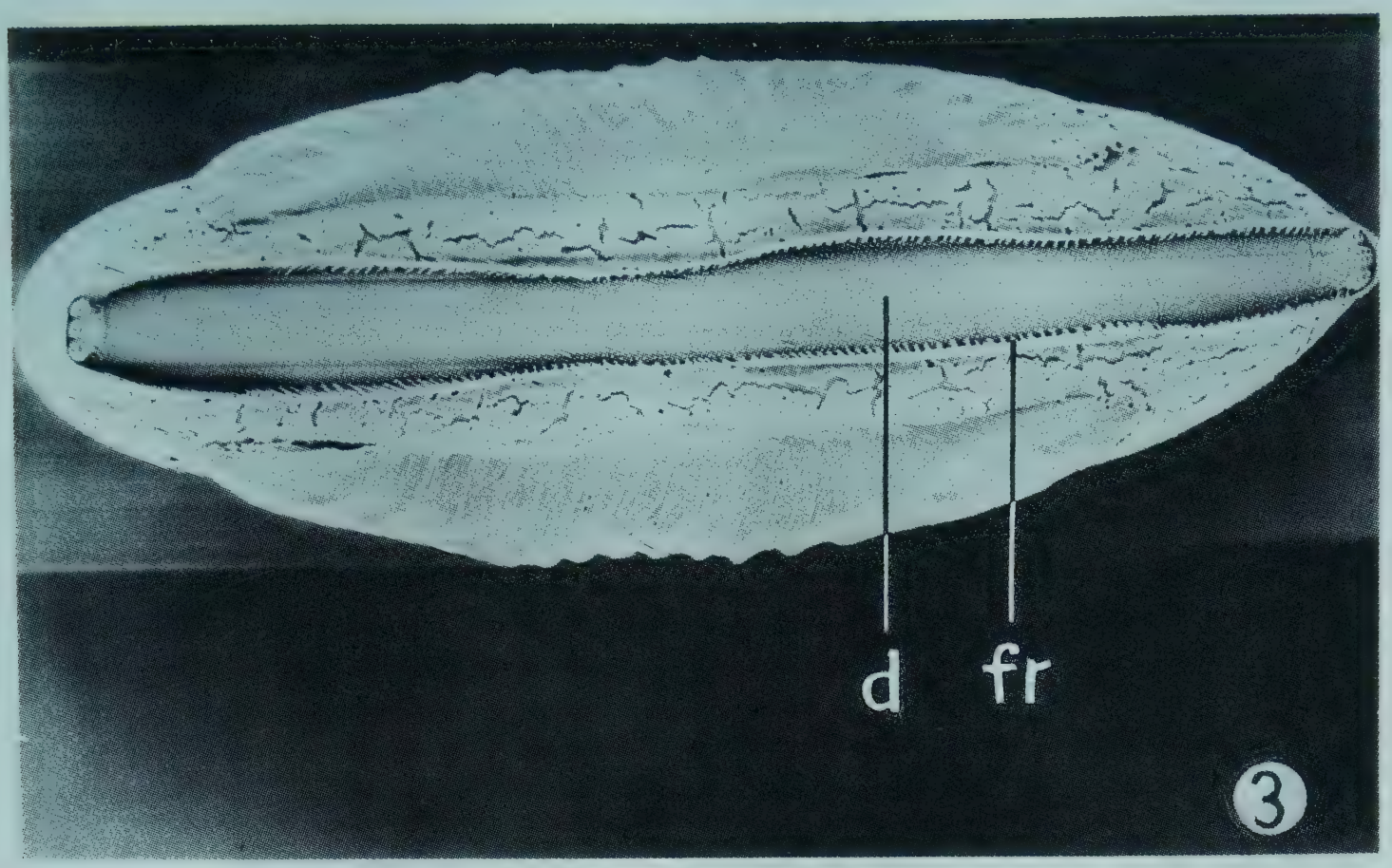


Fig. 5: Ventral view of the egg of species U showing continuous deck (d) surrounded by frill (fr) x 500

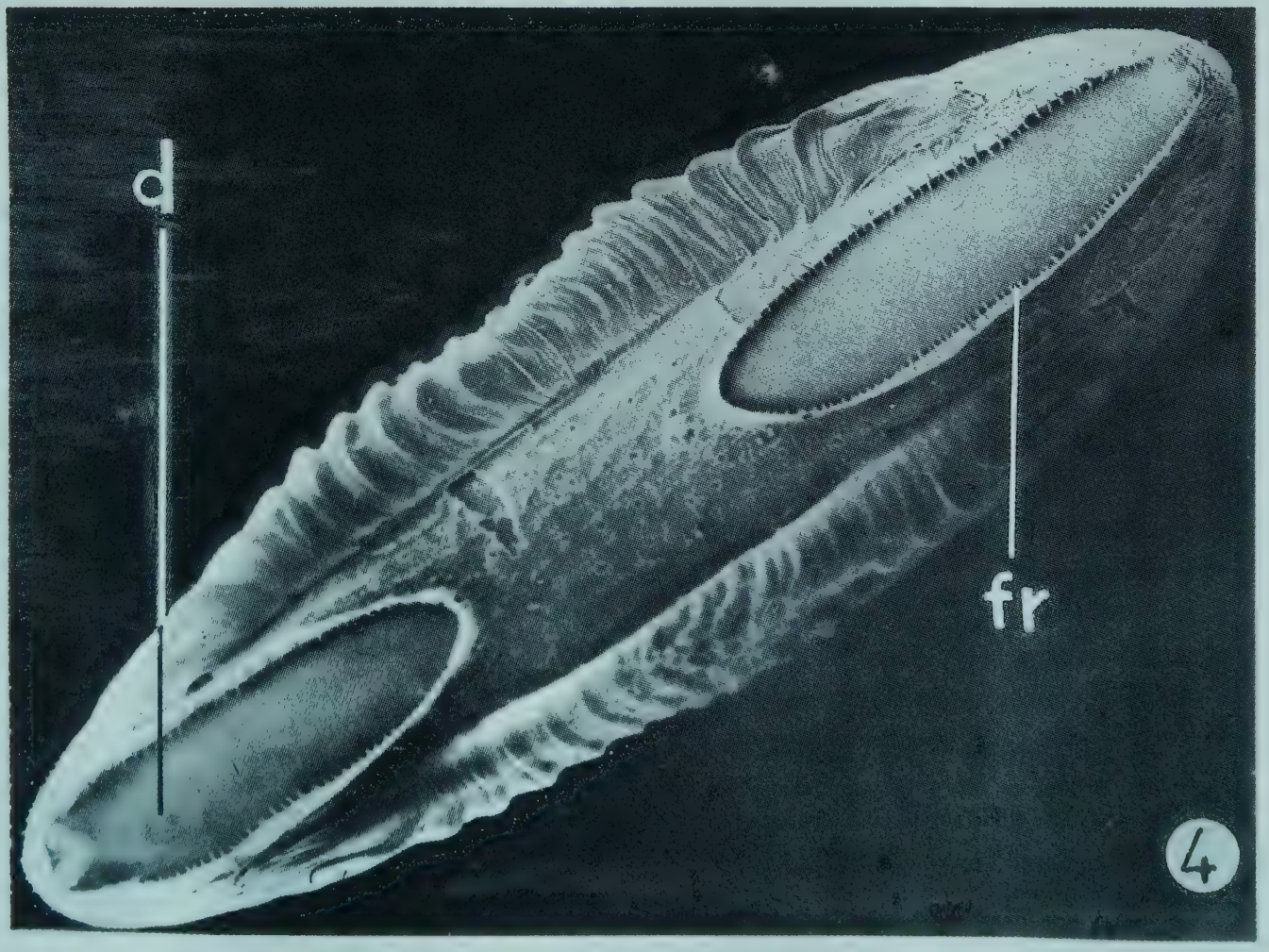


Fig. 6: Alternate form of the egg of species U showing chorion fused in the middle. The deck (d) is divided into two parts each surrounded by frill (fr) in circular fashion

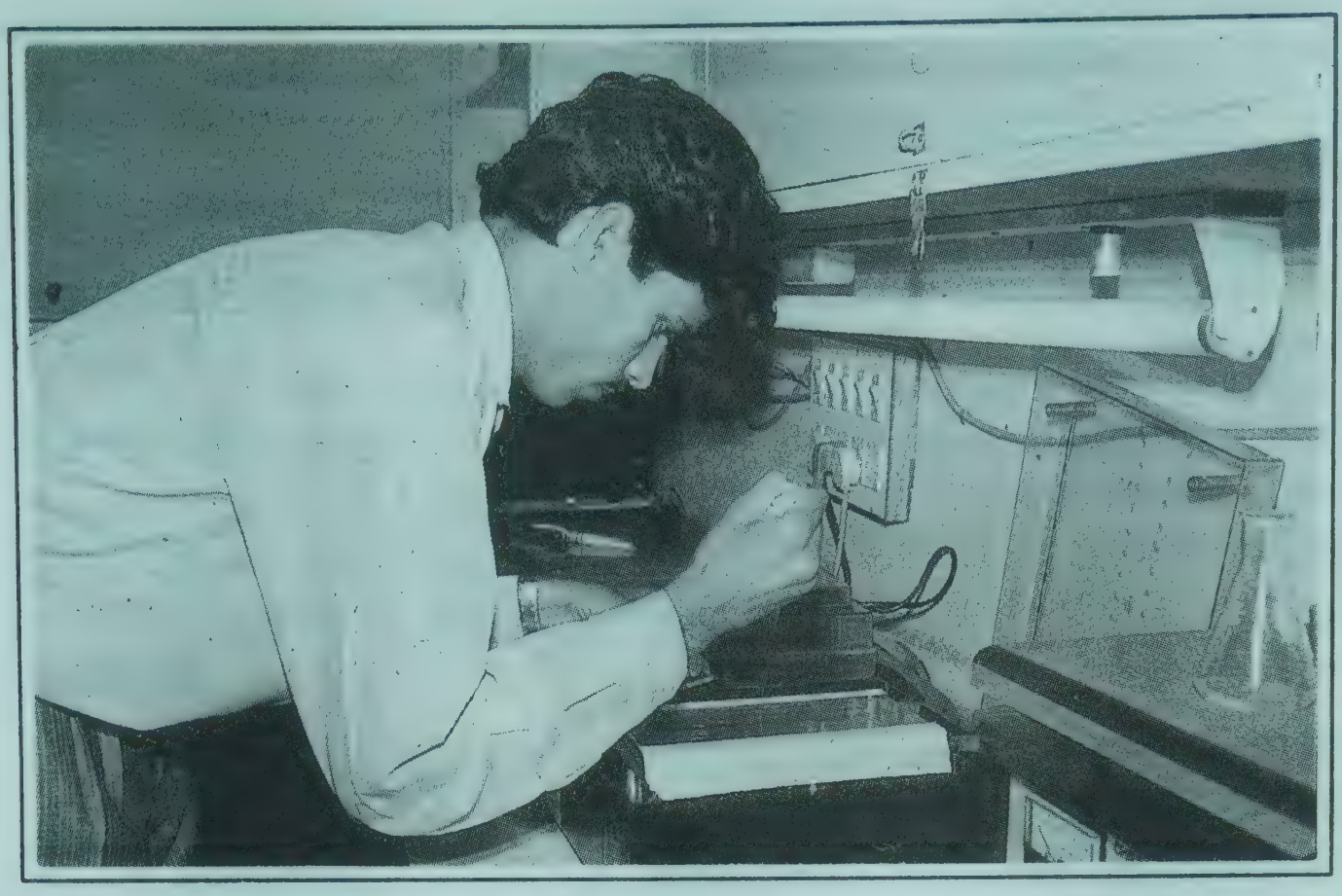
Since this variation is encountered in both the species it does not serve as species diagnostic character and can be regarded as an intraspecific variation. However, in species T majority of the eggs examined had chorion fused in the middle whereas, in species U both the types, the ones with continuous deck and the others with divided deck were found in almost equal proportion.

An. minimus Sibling Species Identification Using Gene Enzyme System

An. minimus is an important malaria vector of North-eastern states of India. Recent studies in China and Thailand undertaken on this species revealed that this is a species complex. So far, species A, B and C have been identified in this species complex. Species A and C have been reported from Thailand and species B from China on the basis of diagnostic enzyme marker, Octanol dehydrogenase (ODH). Isoenzyme studies were carried out on Indian population to study the biological variation and to understand their role in the transmission of malaria. Mosquitoes collected from different districts of Assam have been screened for five different enzyme systems, viz. LDH, MHD, 6-PGD, MPI and ODH (Table 16). Among the enzymes studied 6-PGD and ODH were found to be dimer, MPI monomer and LDH tetramer ; all the enzymes screened were found to be autosomal in nature. Data from progeny of a total of 106 single female culture were screened. On the basis of ODH polymorphisms it may be pointed out that Indian population consists mainly of species A.

Table 16. Enzyme phenotype observed in *An. minimus* from Assam

Enzyme	No. of progeny tested	Enzyme phenotype			Protein configuration
		S	SF	F	
Lactate dehydrogenase (LDH)	75	70	5	–	Tetramer
Malate dehydrogenase (MDH)	48	–	0	48	–
6-Phosphogluconate dehydrogenase (6-PGD)	37	–	4	33	Dimer
Manose phosphate isomerase (MPI)	37	12	4	21	Monomer
Octanol dehydrogenase (OCH)	106	1	9	96	Dimer



Application of samples on a polyacrylamide gel for electrophoresis

Laboratory Simulation to Study the Behaviour of Malathion-Susceptible Gene in *An. stephensi* Populations

A study was carried out on laboratory strains of *An. stephensi* to observe the behaviour of malathion-susceptible gene in the population under variable frequencies of susceptible and resistant genes. For these studies, lines were established in known proportions of resistant and susceptible genes. Prior to the establishment of lines, certain population attributes were determined for resistant and susceptible lines (Table 17). The lethal time, LT_{50} of the homozygous malathion-resistant and - susceptible strains as computed from time-mortality response studies was 464 min. and 4.2 min. respectively. The resistance gene was dominant.

Pre-mated and blood fed susceptible and resistant *An. stephensi* mosquitoes were held in cages in known susceptible and resistance gene frequencies. The frequencies of the resistant and susceptible gene frequencies in the cages were 0.1 : 0.9, 0.2 : 0.8, 0.5 : 0.5, 0.8 : 0.2 and 0.9 : 0.1 respectively. Mosquitoes were maintained in an insectary using the standard mosquito colonization procedures. These lines are considered as parent generation and from F_1 generation onwards the populations were inbred. From F_2 onwards, a random sample of 200 I instar larvae were removed and colonized for the continuation

Table 17. Life-table and developmental attributes of the laboratory strains of malathion-susceptible and -resistant *An. stephensi*

Developmental attributes	Susceptible (SS)	Resistant (RR)
Longevity	21.5 days	25.0 days
Life expectancy	11.26 days	11.76 days
AGE–mean of cohort reproduction	9.42 days	9.34 days
Net reproduction rate (Ro) eggs	167.82*	274.16*
Intrinsic rate of increase (r_m)	0.202	0.259
Gross reproductive rate (m_x) (Total eggs)	564.85*	724.62*

* – Significant difference was found between the two population

Table 18. Susceptible gene frequency in the population in simulation studies of the laboratory strain of *An. stephensi*

Lines	Q_i	Q_n	No. of generation
RR:SS*			
0.9:0.1	0.59	0.86	16
0.9:0.1	0.45	0.88	16
0.8:0.2	0.67	0.77	9
0.8:0.2	0.89	0.97	4
0.5:0.5	0.79	0.99	16
0.5:0.5	0.69	0.97	16
0.2:0.8	0.99	1.00	16
0.2:0.8	0.83	0.99	16
0.1:0.9	0.56	0.98	16

* – Genotype frequency of the population; Q_i – Frequency of the susceptible gene in the F1 generation; Q_n – Final frequency of the susceptible gene in the population (after 'n' no. of generations)

of the respective lines. From each of the generations, 3-day-old adult mosquitoes were exposed to diagnostic dose of malathion as specified by WHO i.e. 5.0% malathion impregnated papers for one hour and 24 hours recovery period. From the per cent mortality in a given generation of the given line the frequency of the susceptible gene in the population was determined.

All the lines were monitored for the susceptible gene frequency for 16 generations. However, cages with the susceptible gene frequency of 0.2 and a cage with 0.9 gene frequency could not be monitored for all the 16 generations. Initial and final frequencies of the susceptible gene in the population in the respective lines is given in Table 18.

In general, among all the lines studied, stabilization of the gene frequency of the resistant and susceptible genes was observed between F_3 - F_7 generations depending on the initial frequency of susceptible gene in the population.

Statistical analysis of the above data is in progress to determine the selection coefficients of the respective lines in given generations taking into consideration associated population attributes to understand the behaviour of malathion-susceptible gene in the population.

Evaluation of Biolarvicides

Comparative efficacy of five Bti formulations against mosquito larvae: Table 19 gives the list of five Bti formulations viz. Bactoculicide (W.P), Bactolarvicide (L.C.), Moskiture (W.P.), BMP-144 2 x (A.S.) and Deltafix 90 (granules), used in this study. Table 20 shows the comparative efficacy of five biolarvicides against III instar larvae of *An. culicifacies*, *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* under laboratory conditions. Among five Bti formulations, Bactoculicide was the most effective biolarvicide against different mosquito species. LC_{50} values of Bactoculicide against *An. culicifacies, An. stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti* were 0.34, 0.13, 0.05 and 0.04 mg/L respectively (Table 20). Of the four vector species *An. culicifacies* was least susceptible to all the five Bti formulations, *Aedes aegypti* was most susceptible to three powder/granule formulations viz. Bactoculicide, Moskiture and Deltafix 90 but against two liquid formulations i.e. BMP-144 2 x and Bactolarvicide, *Culex quinquefasciatus* was more susceptible than *Aedes aegypti*.

Table 19. List of biolarvicides (Bti) formulations and their source

Biolarvicide	Formulation	Source	Supplier
Bactoculicide	W.P.	Ministry of Medical Industry, Russia.	Chemicals International Ltd.
Bactolarvicide	Liquid conc.	–do–	–do–
BMP–144–2x	Aqueous suspension	Becker Microbial Product Inc., USA	Hindustan Insecticide Ltd.
Moskiture	W.P.	Czechoslovakia	M/s. Kejeriwal Enterprizes
Deltafix 90	Granules	Vector Control Research Centre, India.	V.C.R.C.

Evaluation of five Bti formulations against *An. stephensi* and *Cx. quinquefasciatus* in underground basement tanks: Table 21 shows comparative efficacy of five Bti formulations against larvae of *An. stephensi* and *Cx. quiquefasciatus* under field conditions in underground basement tanks. All Bti formulations except Deltafix 90, the slow release granule formulation, produced 100% reduction in the density of III+IV instar larvae of *An. stephensi* within 24 hours when they were applied @ 0.05 and 0.25 g/sq m. However, after one week there was no reduction in the density of III+IV instar larvae @ 0.05 g/sq m, but at 0.25 g/sq m, Bactoculicide still produced 100% reduction in the density of III+IV instar larvae, while the other biolarvicides showed variable impact. Deltafix 90 granules @ 5 g/sq m produced only 25-54% reduction in 24 hour and 80-100% reduction after 48 hours. After one week the per cent reduction in III+IV instar *An. stephensi* larvae with Deltafix 90 granules was highly variable (0-90%). Three Bti formulations namely Bactoculicide, Bactolarvicide and BMP-144-2x produced 100% reduction in the density of III+IV instar of *Culex* larvae @ 0.25 g/sq m and 97-99% @ 0.5 g/sq m but Moskiture produced only 75-79 % reduction at this dosage. After one week Bactoculicide, BMP-144 2x and Bactolarvicide still produced 89-100% reduction @ 0.05 g/sq m but Moskiture at the same dosage produced no reduction (0%) in the density of III+IV instars. Deltafix 90, the slow release granule formulation produced only 62-77% reduction after 24 hour but after one week 89-93% reduction was noticed.

Table 20. Comparative efficacy of five Bti formulations against III instar larvae of *An. culicifacies, An. stephensi, Cx. quinquefasciatus* and *Aedes aegypti*

Mosquito spp.		Lethal concentration in mg/l				
	LC value	Bactoculicide (W.P.)	Bactolarvicide (Liquid conc.)	BMP-144-2x (Aqueous susp.)	Moskiture (W.P.)	Deltafix 90 (Granules)
An. culicifacies	LC_{50}	0.347 (0.28-0.42)	0.772 (0.64-0.94)	2.54 (2.21-2.92)	0.49 (0.36-0.68)	>320
	LC_{90}	1.85	3.69	6.24	4.0	–
An.stephsnsi	LC_{50}	0.13 (0.099-0.18)	0.52 (0.47-0.58)	0.81 (0.71-0.93)	0.21 (0.16-0.27)	<20
	LC_{90}	0.5	0.88	2.0	0.65	–
Cx. quinquefasciatus	LC_{50}	0.056 (0.042-0.076)	0.052 (0.04-0.06)	0.065 (0.075-0.096)	0.16 (0.15-0.17)	>40
	LC_{90}	0.38	0.13	0.19	0.54	–
Ae. aegypti	LC_{50}	0.04 (0.03-0.05)	0.16 (0.15-0.19)	0.6 (0.5-0.7)	0.1 (0.09-0.11)	<20
	LC_{90}	0.14	0.36	1.67	0.22	

Figures in parantheses indicate 95% confidence limit

Table 21. Efficacy of five Bti formulations against larvae of *An. stephensi* and *Cx quinquefasciatus* in basement tanks under field conditions

Biolarvicide	Dose (gm^2)	Pre-treatment density (III + IV instar)	*An. stephensi*			Pre-treatment density (III + IV) instar	*Cx. quinquefasciatus*		
			% reduction (days post-treatment)				% reduction (days post-treatment)		
			1	2	7		1	2	7
Bactoculicide	0.25	2.3	100	100	100	29.6	100	100	89
	0.05	2.4	100	100	5	42.8	97	100	100
Bactolarvicide	0.25	1.6	100	100	43	5.4	100	100	100
	0.05	2.0	100	100	0	20.4	98	100	94
BMP-144-2x	0.25	1.2	100	100	74	10.5	100	100	100
	0.05	1.5	100	100	0	18.3	99	100	100
Moskiture	0.25	2.8	100	100	93	13.5	75	96	81
	0.05	4.1	100	96	0	14.9	79	66	0
Deltafix 90	5.0	12.2	25	80	90	412	77	77	89
	5.0	17.0	54	100	0	17.0	62	94	93

Post-treatment monitoring of larvae

Effect of Sunlight on the activity of Spherix (*B. sphaericus*): In addition to environmental factors such as temparature and water pH, which were earlier reported to influence the larvicidal activity of biolarvicides, the impact of sunlight on the larvicidal activity of Spherix was studied. The effect of exposure to bright sunlight on the larvicidal activity of Spherix was determined using two sets of plastic bowls containing different concentrations of biolarvicides. One set was kept in shade and the other set was placed in direct sunlight for 6 h. After 6 h both the sets were brought to laboratory and bioassay tests were carried out using III instar *Culex quinquefasciatus* larvae. Table 22 shows the influence of exposure to direct sunlight on the % larval mortality of *Culex quinquefasciatus* treated with different concentrations of the Spherix (*B. sphaericus*). The Table 22 clearly shows higher percentage mortality in bowls that were kept in shade than those kept in direct sunlight.

Evaluation of Neem (*Azadirachta indica*) Products

Effectiveness of neem oil as larvicide: In order to minimize the use of synthetic chemical insecticides against mosquitoes, studies were undertaken to evaluate indigenous, economic and environmentally compatible neem (*Azadirachta indica*) products against mosquito lar-

Table 22. Effect of exposure to sunlight on the larvicidal activity of Spherix against *Cx. quinquefasciatus*

Conc. mg/l	Exposed to sunlight % mortality*	Not exposed to sunlight % mortality*
0.04	51	96
0.02	22	93
0.01	22	66
0.005	26	64
0.0025	27	52
Control	14	11

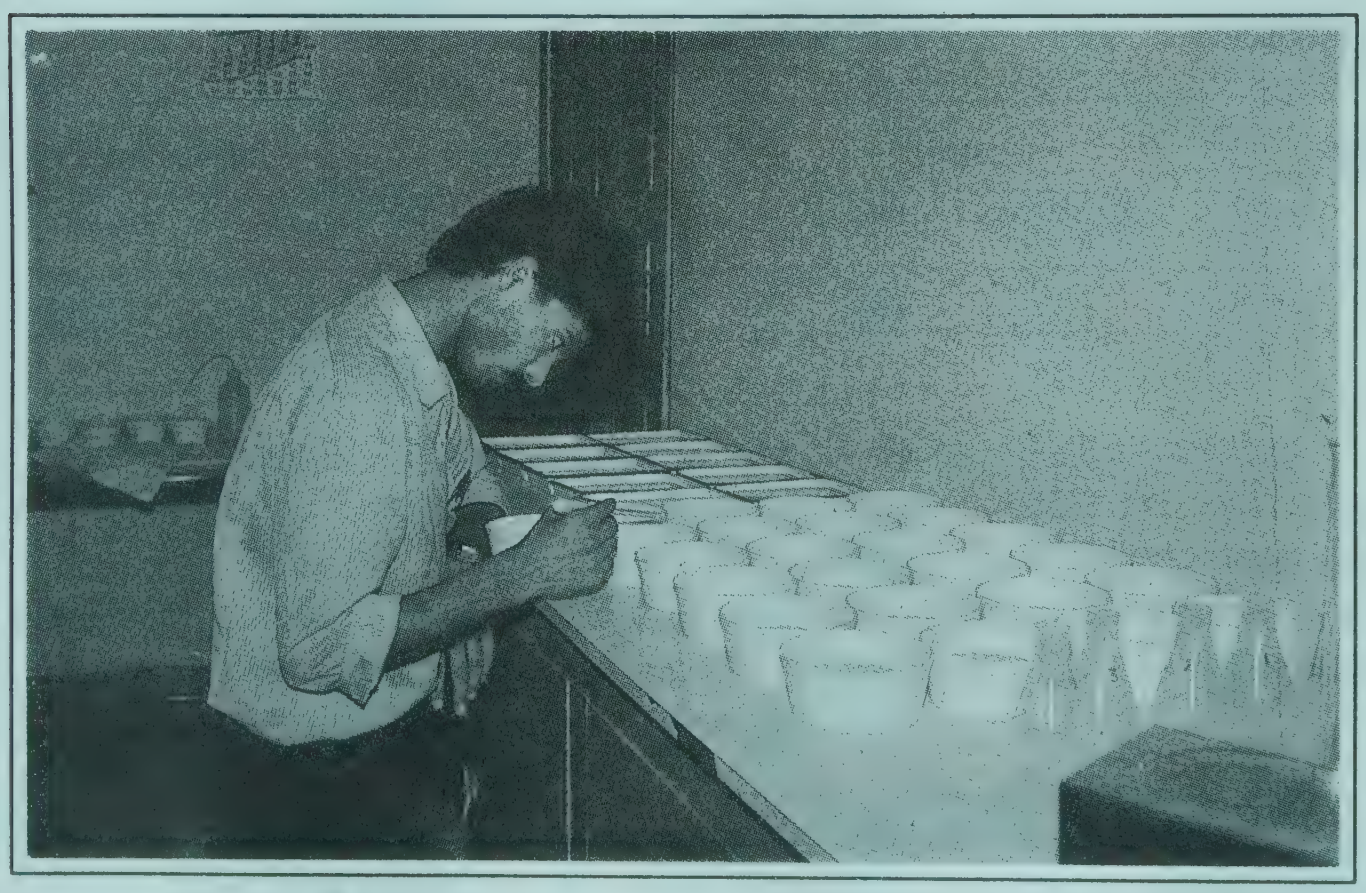
*48 h after exposure/non-exposure to sunlight

Table 23. Efficacy of neem products against IV instar mosquito larvae

Products	Source	*An. stephensi*		*Cx. quinquefasciatus*		*Ae. aegypti*	
		EC50	EC90	EC50	EC90	EC50	EC90
Neem oil	Unjha Ayurvedic Pharmacy	0.0022	0.007	0.0092	0.042	0.0158	0.062
Neemark E.C.	West-Coast Herbochem	0.0005	0.0026	0.0025	0.0089	0.0102	0.0308
NSK-Metha-nolic extract	West-Coast Herbochem	0.0214	0.054	0.0176	0.058	0.0554	0.1974
Achook powder	Godrej	0.0145	0.0147	0.0390	0.0892	0.0481	0.2011
Neemrich–I E.C.	NCL	0.0038	0.0114	0.0111	0.0288	0.0134	0.0487
Neemrich–II E.C.	NCL	0.0026	0.0080	0.0023	0.0062	0.0055	0.0104

vae. Thus six neem products viz neem oil marketed by Unjha Ayurvedic Pharmacy, Neemark E.C. and NSK metamolic extract supplied by West Coast Herbochem Industries, Achook powder, marketed by Godrej Soap Industries Pvt. Ltd. and Neemrich I and II supplied by National Chemical Laboratories, were tested for their larvicidal

impact against *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. In general, the different neem products had poor acute toxicity against larvae but some of these products displayed effective insect growth inhibition activity particularly against *Anopheles stephensi*. Table 23 shows the comparative efficacy of six neem products against larvae of *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. Of the six products tested, Neemark E.C. was most effective against *An. stephensi* (LC_{50} = 5 ppm) while Neemrich II was comparatively more effective against *Culex quinquefasciatus* and *Aedes aegypti* than other products. Neemark was further evaluated under simulated field conditions against larvae of *An. stephensi* and *Culex quinquefasciatus*. Simulated trials in enamel trays revealed complete inhibition of adult emergence in case of *An. stephensi* at 50 ppm concentration, while there was upto 80% reduction in adult emergence in case of *Culex quinquefasciatus* (Table 24). Evaluation of Neemark under field conditions revealed only partial control of *An. stephensi* and *Culex quinquefasciatus* (Table 25).



Testing the bioefficacy of neem products against mosquito larvae

Effectiveness of neem oil against mosquitoes as a topical applicant: Yet in another experiment neem oil was mixed at 0.5, 1 and 2% strength in coconut oil. Three to five ml of this oil was applied on the face, arms and legs of the volunteers between 1745 to 1800 h and simultaneously

Table 24. Laboratory evaluation of Neemark against the larvae of *An. stephensi* and *Cx. quinquefasciatus*

Concentration ppm	*An. stephensi*		*Cx. quinquefasciatus*	
	1	2	1	2
100	0 (100)	0 (100)	19 (82.4)	13 (86.1)
50	2 (99)	0 (100)	26 (75.9)	22 (76.6)
20	118 (44.6)	81 (52.1)	92 (14.8)	71 (24.5)
10	146 (31.45)	62 (63.3)	86 (20.4)	66 (29.7)
Control	213 (–)	169 (–)	108 (–)	94 (–)

1 – pupal yield (% reduction); 2–No. emerged (% reduction); % reduction was based on control; 100 larvae of each instar (I to IV) were added in enamel trays before treatment

Table 25. Field evaluation of Neemark* against breeding of *An. stephensi* and *Cx. quinquefasciatus* in UGT/basements

Days	Control[1]		Treated[2]		Pupae yield	
	Anopheles	*Culex*	*Anopheles*	*Culex*	Control	Treated
0	1.8	40.6	3.1	36.1	2.13	5.45
3	2.11	35.7	1.8	14.5	4.2	1.7
7	2.81	18.7	0.8	6.15	4.0	1.2
14	1.86	16.4	0.5	6.25	2.47	0.3
21	7.0	11.0	0.7	24.2	1.56	1.3
28	3.5	12.3	2.7	7.6	1.6	1.2
35	1.1	2.0	1.7	8.5	1.6	2.0

*Neemark was applied @ 5 ml/m^2; 1 – Average of three replicates; 2 – Average of four replicates

volunteers applied coconut oil alone for comparison (control). Mosquitoes were collected from 1800 to 0600 h. For all night collection, a volunteer served as bait from 1800 h to 1200 h and he was replaced by another volunteer till 0600 h. For each dosage of neem and coconut oil, volunteers occupied one room separately. They were asked to lie on a cot inside a human dwelling unit. Mosquitoes land-

ing on the exposed body were collected by an insect collector (untreated normal) with the help of a suction tube using a flash-light. Volunteers, insect collectors and experimental rooms were randomized each night and collections were made on the treated and control volunteers concurrently on 10 different nights. Results of these observations and percentage protection from the biting of mosquitoes are given in Table 26. Per cent protection from man mosquito contact was calculated.

Table 26. Results of neem oil mixed in coconut oil and applied on the exposed body parts of volunteers on the biting rate of mosquitoes, Sept–Oct, 1992

Neem oil (%) mixed in coconut oil	Total number of mosquitoes landed on indoor human baits in 10 nights					
	An. culicifacies			Total anophelines		
	Neem oil	Control	% protection	Neem oil	Control	% protection
0.5	11	74	85.1	14	87	83.9
1	10	167	94.0	30	408	92.6
2	0	48	100.0	0	144	100.0

Results revealed that neem oil produced a strong repellent action on *An. culicifacies*, and other anopheline mosquitoes causing nuisance, even at as low as 0.5 and 1% concentration. At 2% concentration there was no bite of *Anopheles* mosquitoes and the protection provided was 100% for a period of 12 h. Further studies are indicated to evaluate its efficacy against different vector species.

Protection from mosquitoes by burning neem oil in kerosene lamp: Efforts were continued to develop low-cost, indigenous appropriate personal protection measures for poor socio-economic group living in remote and inaccessible areas. Last year esbiothrin and kerosene lamps were evaluated for repellent action of lamp smoke and found effective in preventing the bite of the adult *An. culicifacies*, a principal vector of malaria in northern plains of the country (MRC, Annual Report, 1992). This year results of tests with neem oil extracts from the seeds of *Azadirachta indica* as deterrent in interrupting the transmission of malaria was studied.

Table 27. Results of burning kerosene oil lamps containing neem oil on the density of resting mosquitoes in living rooms and biting rate on volunteers

Sl.no.	Neem oil (%) in kerosene oil	Resting mosquito collection and landing on human baits											
		An. culicifacies			Total anopheline			*Culex* spp.			Total mosquitoes		
		E	C	% prot.	E	C	% prot.	E	C	% prot.	E	C	% prot.
1.	0												
	Living room	220	343	35.8	408	643	36.5	1919	3162	39.3	2327	3807	38.8
2.	0.05%												
	Living room	28	362	92.2	55	747	92.6	1015	2807	63.8	1070	3554	69.8
	Human bait	11	47	76.5	19	75	74.6	545	1917	71.5	564	1992	71.6
3.	0.1%												
	Living room	28	711	96.0	70	1105	93.6	1961	7087	72.3	2031	8192	75.2
	Human bait	4	24	83.3	8	45	82.2	1343	4824	72.1	1351	4869	75.2
4.	0.5%												
	Living room	6	257	97.6	35	543	93.5	400	2019	80.1	435	2562	83.0
	Human bait	7	45	84.4	28	146	80.8	271	1210	77.6	299	1256	77.9
5.	1%												
	Living room	10	1013	99.0	106	1838	94.2	896	4711	80.9	1002	6549	84.6
	Human bait	0	41	100.0	0	108	100.0	569	2729	79.1	569	2837	79.9
6.	2%												
	Living room	4	536	99.2	48	2059	97.6	216	1610	86.5	264	3669	92.8
7.	4%												
	Living room	6	590	98.9	90	2357	96.1	253	1053	75.9	343	3410	89.9

Note : From a total of 10 replicates at each dosage in experiment and control separately mosquito collection were made from 1800 - 0600 hrs; S.No. 1 E = Kerosene lamp; C = No lamp; S.No. 2-7 E = Neem oil mixed in kerosene; C = Kerosene alone; % Prot. = % protection

Studies were carried out in Ramgarh (Population, 1137) and Jadaonpur (Population, 693) villages located in Dadri and Dhaulana primary health centres respectively, District Ghaziabad, U.P. at a distance of about 30 kms in south-east of Delhi. These villages are endemic for malaria. In these villages mosquito densities were high and *An. culicifacies* is the principal vector of malaria. Neem oil locally marketed by Unjha Ayurvedic Pharmacy, Gujarat as Ayurvedic medicine to purify blood and for cure of skin ailments was purchased. Neem oil in liquid form is sold as purified product. It has unpleasant odour and can be mixed in kerosene oil.

A tin lamp of 100 ml capacity with cotton wick of 1mm diameter and a regulator was used. Lamps with neem oil in kerosene or kerosene alone were burnt all night from 1800 - 0600 h. Single lamp was used in one room. For this study living rooms of 3 to 3.2 m width and 3.8 to 4.1 m length were selected. Room bias was eliminated by rotation between experimental and control rooms. Lamps were placed in a secure place on a wall in the centre at the height of 1.5 m.

Mosquitoes were collected from living rooms at hourly interval for 15 minutes in the same rooms but on different dates. Man biting rate of mosquitoes was studied on human volunteers. Arms, legs and face of the volunteers were exposed to serve as bait in lying position on a cot for 6 h. Bait was replaced after 6 h and therefore, each night two baits were used in one room. All mosquitoes landing on baits were collected by an insect collector, with the help of suction tube and flash light. Insect collectors were changed every hour. For comparison (control) mosquitoes were collected from rooms with and without lamp and also from rooms with lamp containing neem oil in kerosene and kerosene alone. A total of 10 collections were made at each dosage in the rooms and 10 on human volunteers. Mosquitoes collected from the field were identified specieswise, pooled and tabulated and percentage protection was calculated by using following formula :

$$\% \text{ protection} = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

Table 27 gives the result of burning neem oil in kerosene lamp on the mosquitoes present in living rooms and those found landing on the exposed body parts of human volunteers. Results revealed that kerosene lamp alone (S.No. 1) reduced mosquito population in rooms

by 35-40% which may be the effect of light and smoke from kerosene. When neem oil was added in kerosene it reduced the resting mosquito population in living rooms. The biting rate also reduced. At as low as 0.05% concentration of neem oil the deterrent action was pronounced both on the resting mosquito population and the biting rate of *Anopheles* and *Culex* mosquitoes on volunteers. However, the action was more pronounced on *Anopheles* than on *Culex*. There was >90% protection from *Anopheles* and >60% from *Culex* resting mosquitoes whereas, on human volunteers the protection was about 70% from *Anopheles* and *Culex*. Increased neem oil concentration of 0.1% did enhance protection but marginally in landing rate of mosquitoes. Increased dosage of 0.5% had essentially the same impact as 0.1% but further increase to 1% was highly effective as deterrent to the entry of mosquitoes in rooms and biting rate on volunteers. Protection provided from resting mosquitoes was found to be, *An. culicifacies* 99%, all anophelines 94.2%, *Culex* spp. 80.1% and 83% from all mosquitoes. No anopheline mosquito landed on human bait at 1% neem oil although protection from *Culex* landing was 79%. Therefore, 1% neem oil in kerosene can provide 100% protection from the bite of *Anopheles* mosquitoes and substantially reduce mosquito nuisance. Further increase in neem oil concentration to 2 to 4% was not found effective as there was problem in smooth burning of wick. During the study period volunteers and insect collectors were enquired of any adverse reaction on their health. There was no complaint of ocular irritation, odour, cough, rash, respiratory or any other problem.

Village-scale trial with neem oil in kerosene lamps: In view of encouraging results, a field trial was carried out in Beel Akbarpur village (Population, 962) to evaluate the operational feasibility of neem mixed kerosene oil lamp smoke in repelling adult *An. culicifacies* and its impact in interrupting the transmission of malaria.

Since *An. culicifacies* is basically a zoophilic species, therefore, lamp was burnt only in living room. 1% neem oil was mixed in kerosene oil at distribution centre and supplied to inhabitants along with the standard tin lamps. They were asked to burn the lamp from 1800 to 0600 h everyday in each living room without interruption. Anandpur village (Population, 1145) was taken as control, where only kerosene lamp was lighted in each living room. The pre-density of the vector species and incidence of malaria were comparable in experimental and control village.

Entomological and epidemiological data were collected to monitor the density of *An. culicifacies* in human dwellings and cattlesheds

along with man-mosquito contact. Door-to-door surveillance was carried out and presumptive treatment was given to all fever cases. Microscopically *P. vivax* positive cases were provided radical treatment in both the villages. *P. falciparum* positive cases were provided 1500 mg, chloroquine in 3 days.

Results of field trial revealed that smoke from burning neem and kerosene oil lamp has successfully diverted adult *An. culicifacies* to cattleshed rooms where lamp was not burnt. However, diversion in control village was not to the same extent where only kerosene lamp was burnt. The pre-experiment density of 64.85 of vector species was reduced to 13.25 within three months period and remained marginally low in subsequent months. Withdrawal of kerosene lamp with neem oil from human dwellings gradually resulted in reversion of adult density (Table 28).

The impact of smoke was also evident on incidence of malaria. During post-experiment period only 1 *Pv* in the month of June was detected in Beel Akbarpur village as against 6 *Pv* and 5 *Pf* cases in Anandpur village where only kerosene lamp was used in living rooms. Further trials in endemic and tribal areas against various vector species are indicated or being planned.

Effectiveness of neem oil mats in repelling mosquitoes: A variety of mats and coils are marketed in India to avoid mosquito bites. Mats on heating and coils on burning release vapours that repel mosquitoes. Neem has multiple action and it is a good insect anti-feedant as tested against agricultural insects. We have, therefore, evaluated the effectiveness of neem oil in repelling mosquitoes in comparison with mats containing allethrin.

Rithala village situated on the outskirts of west Delhi was selected to carry out studies in November, 1992 when *Anopheles* densities were low and *Culex quinquefasciatus* densities were high. Neem oil was diluted in acetone to give 5% and higher concentrations. Initially experiment was conducted with different concentrations of neem oil soaked mats to select the dosage for further studies. Experiment revealed that neem oil was more effective at low concentrations, although it provided protection at all dosages used. We, therefore, selected 5 and 10% neem oil for further studies. A card board mat of 35mm x 25mm x 3 mm size was soaked in one ml diluted neem oil.

Table 28. Entomological and parasitological indices of Beel Akbarpur (experimental) and Anandpur (control) village, District Ghaziabad

Month	Average MHD of *An. culicifacies*									
	Beel Akbarpur (E)		Anandpur (C)		Beel Akbarpur (E)			Anandpur (C)		
	HM	CS	HM	CS	BS	*Pv*	*Pf*	BS	*Pv*	*Pf*
Pre-experiment										
Apr 92	79.7	131.0	74.8	105.5	11	0	0	8	0	0
May	50.0	74.5	45.2	69.0	7	3	0	17	3	0
Average	64.85	102.75	60.0	87.25	18	3	0	25	3	0
Post-experiment										
Jun 92	36.3	68	67.8	103.7	7	1	0	10	2	0
Jul	34.2	79.75	97.5	132.2	8	0	0	20	1	0
Aug	23.7	79.2	62.0	83.0	5	0	0	8	0	0
Sept	13.25	35.75	28.87	46.25	16	0	0	14	1	2
Oct	9.37	45.0	16.12	43.1	5	0	0	9	1	0
Nov	6.0	37.7	11.2	42.0	4	0	0	4	1	1
Dec	5.25	12.7	10.8	19.2	3	0	0	5	0	1
Jan 93	3.25	22.7	12.0	21.0	4	0	0	5	0	0
Feb	4.25	28.7	15.2	29.2	5	0	0	6	0	0
Mar	5.1	32.2	16.2	30.7	5	0	0	9	0	1
Apr	22.3	36.2	29.0	44.7	7	0	0	6	0	0
Average	14.81	43.4	33.33	54.9	69	1	0	96	6	5
Follow up:										
May 93	35.5	55.2	31.1	45.0	4	0	0	3	0	0
Jun	48.1	68.7	44.5	66.2	5	0	0	4	0	0
Jul	52.3	70.2	42.5	69.2	4	0	0	3	0	0
Aug	46.1	72.5	43.2	70.2	19	1	0	9	1	0
Sept	48.5	70.2	47.6	66.2	21	1	0	6	1	1
Oct	45.2	68.7	44.1	61.7	21	0	2	6	0	0
Nov	32.2	50.5	28.0	39.7	13	0	0	8	0	0
Dec	14.2	28.2	13.3	27.2	11	0	0	7	0	0
Average	40.2	60.5	36.7	55.9	98	2	2	46	2	1

HM: Human and mixed dwellings, neem oil lamp was burnt w.e.f. 1 June 1992; neem oil lamp was discontinued w.e.f. April 1993

An electric device for heating the mat marketed by the trade name of 'Jet' was used. To evaluate the repellent action of neem oil mats, two concurrent controls were run, one with 'AL-4' mat and a second without a mat. One mat each was used in a room of size 3m x 4m from 1750 to 0500 h. Jet with mat were placed at the centre of a wall. Control and experimental rooms were also rotated to avoid sampling errors. Similarly, insect collectors and human baits were rotated. Mosquitoes that came in contact with the volunteer (probing was not allowed) were collected all night from 1750 - 0500 h. At 0515 h all resting mosquitoes in the room were collected by 2 insect collectors for 30 min in each.

Results revealed that man biting rate of *Cx. quinquefasciatus* for one night based on 10 nights collection was 129.7 $\pm$ 74.8 with NO-5 (5%) mat, 124.9 $\pm$ 55.8 with NO-10 (10%) mat and 187.6 $\pm$ 90.3 with AL-4 mat as compared to 729.8 $\pm$ 311.8 with mat. The difference was not statistically significant between NO-5 and NO-10 mats ($P>0.4$) but all other comparisons were significant i.e. NO-5 and AL-4 ($P<0.01$); NO-5 and no mat ($P<0.005$); NO-10 and AL-4 ($P<0.05$); NO-10 and no mat ($P<0.005$) and AL-4 and no mat ($P<0.005$). In comparison to the control, percentage reduction in all night biting rate was 82.3% in NO-5, 83% in NO-10 and 74.3% in AL-4 mats (Table 29).

To check the effect of heating of neem oil mats in prohibiting the mosquito entry in rooms, the entry was monitored by the collection of resting mosquitoes at 0515 h in living rooms where the mats were used. Total *Anopheles* mosquitoes collected in 10 nights were 2 with NO-5, 3 with NO-10, 8 with AL-4 and 95 in control. Reduction from the control was 98, 97 and 91% respectively (Table 30). t-test at 5% level showed that the difference in NO-5, NO-10 and AL-4 was not significant, however, when compared with control it was significant i.e. NO-5 and control ($P<0.05$); NO-10 and control ($P<0.01$) and AL-4 and control ($P<0.01$). In the same rooms *Cx. quinquefasciatus* collected in 10 nights were 78 with NO-5, 109 with NO-10, 142 with AL-4 and in the control 212. Reduction from the control was 63, 49 and 33% respectively (Table 30). The effect was more pronounced in *Anopheles* i.e.>90% mosquitoes were repelled. In repelling mosquitoes from landing on baits and entry into the room neem oil produced better results than allethrin mats. Therefore, neem oil can be a good substitute for pyrethroids in coils and mats. Because of safety, good repellent action, low cost and easy availability, 5 to 10% neem oil mats should be preferred to prevent bites of mosquitoes and other haematophagous insects.

Table 29. *Culex quinquefasciatus* biting on human volunteers in rooms with mats treated with neem oil (NO) and allethrin (AL) mats

Time	NO-5 Mean±SD	t test with control	NO-10 Mean±SD	t test with control	AL-4 Mean±SD	t test with control	Control Mean±SD
1730–1800	7.88 ± 6.69	3.44	4.2 ± 3.99	3.97	13.1 ± 9.46	2.81*	39.1±23.86
1800–1900	13.3 ± 8.74	4.47	9.5 ± 6.04	4.90	22.7 ± 9.79	4.34	71.0 ± 38.61
1900–2000	16.7 ± 10.13	5.26	11.1 ± 8.12	6.53	25.7 ± 14.19	4.48	79.6 ± 36.66
2000–2100	14.1 ± 7.08	5.17	12.7 ± 7.68	5.42	25.2 ± 7.49	4.12	84.1 ± 43.46
2100–2200	13.6 ± 9.44	7.12	15.1 ± 8.14	8.24	21.9 ± 7.69	5.95	61.5 ± 22.11
2200–2300	12.4 ± 11.67	5.31	10.2 ± 5.21	5.87	15.1 ± 10.19	5.36	60.3 ± 28.25
2300–2400	14.2 ± 11.68	4.47	12.5 ± 7.03	5.25	14.9 ± 10.05	4.51	64.9 ± 33.35
2400–0100	10.2 ± 9.26	4.52	11.5 ± 5.73	4.98	12.9 ± 7.96	4.56	64.2 ± 34.95
0100–0200	10.4 ± 7.51	5.26	11.3 ± 5.55	5.06	10.1 ± 10.95	5.86	69.4 ± 33.25
0200–0300	8.5 ± 7.42	5.06	9.8 ± 5.36	5.76	11.5 ± 12.28	5.77	57.9 ± 26.40
0300–0400	5.8 ± 5.46	6.37	10.9 ± 4.72	5.69	9.1 ± 9.22	6.75	46.4 ± 20.67
0400–0500	3.5 ± 3.11	4.08	6.1 ± 3.56	3.66	5.4 ± 4.96	3.77	31.5 ± 19.33
Mosquitoes collected (average)	129.00		124.00		187.00		729.00
% reduction from control	82.3		83		74.3		–

Note: Mosquito densities in experimental rooms comapred with control were statistically significant at 5% and 1% except marked with estrisk 1%; Mean ± SD represents average of 10 night collection

Table 30. Collection of indoor resting mosquitoes in room with mats

Nov' 1992 dates	*Anopheles*				*Culex*				Total			
	NO-5	NO-10	AL - 4	Control	NO-5	NO-10	AL - 4	Control	NO-5	NO-10	AL - 4	Control
06	0	2	3	36	12	5	24	14	12	7	27	50
10	1	7	4	17	6	6	10	13	7	13	14	30
12	1	0	2	13	8	17	11	21	23	32	27	34
14	0	0	0	8	8	11	16	25	8	11	16	33
17	0	0	0	3	4	4	7	21	4	4	7	24
19	0	0	0	3	11	9	11	23	11	9	11	26
21	0	0	0	3	9	10	8	19	9	10	8	22
24	0	0	1	5	9	18	26	25	9	18	27	30
26	0	0	0	3	4	16	14	20	4	16	14	20
28	1	0	0	4	7	13	15	34	8	13	15	38
10 nights	2	3	8	95	78	109	142	212	95	133	166	307
% reduction from control	98	97	91	-	63	49	33	-	69	57	46	-

t test at 5% showed significant difference with control in *Anopheles, Culex* and total mosquitoes

Report of the MRC-ISRO Collaborative Project on Feasibility of using Satellite Data to Identify and Monitor Breeding Potential of Mosquitoes in Selected Sites of Delhi (NCT)

Mosquitoes not only create nuisance they are also responsible for transmission of diseases like malaria, filariasis, japanese encephalitis (JE), dengue, dengue-haemorrhagic fever (DHF) etc. in the country. Malaria is still considered a serious public health problem in India and government is spending major part of the health budget to control malaria in both rural and urban areas. In spite of this, the disease is still prevalent and about 2 million cases are recorded annually with progressive trend of falciparum malaria which accounts for 30-40% mortality in complicated cases. Malaria is transmitted by the female anopheline mosquitoes bites. There are about 57 species of anophelines in India but only 9 are considered vectors of malaria. Immature stages of mosquito are developed in aquatic environment while adults thrive in terrestrial environment. Control operations are directed either against immatures or adults. Different types of surveys are required to identify mosquito breeding sites to avoid wasteful expenditure in control operations. Aerial surveys are one of the best solution but its prohibitive cost does not allow mosquito control agencies to update information on breeding sites which keep changing every season. Similarly, manual ground surveys are time consuming, tiring and of limited use as the data can not be utilized for any other purpose. Recent progress in space research particularly after the launch of IRS-IA satellite in the orbit has opened a new era in monitoring earth surface and provide images on land use features including major water bodies at an interval of 21 days. These images are processed and identification of land use class is possible with the help of computer and ground truths. An attempt is therefore made to explore the feasibility of using satellite data for identification of water bodies and prevalence of species in ecosystem and its correlation with vegetation, human settlement, type of water bodies and plantation. Results of Okhla and Hindon barrage are discussed in this report.

Six study sites in and around Delhi (UT) were selected in collaboration with Indian Space Research Organization (ISRO), Bangalore to examine the feasibility of mapping breeding places using remote sensing techniques. Study sites selected were (i) Nazafgarh drain (ii) Bhalaswa lake (West Zone, MCD) (iii) Sanjay lake (iv) Seelampur lakes (Shahdra Zone, MCD) (v) Okhla and (vi) Hindon barrage in District Ghaziabad (U.P.). These sites represent varying topogra-

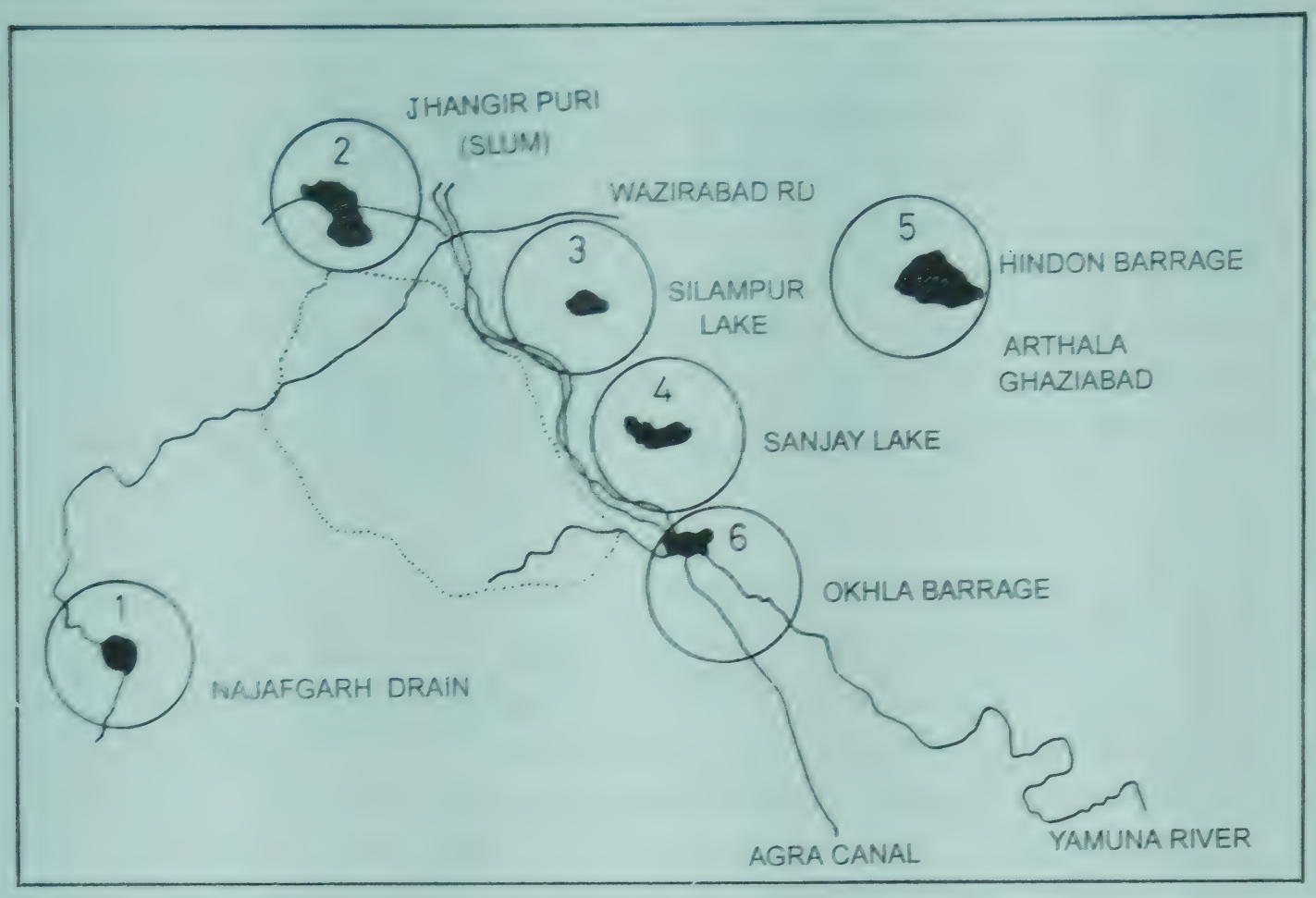


Fig. 7: Map showing location of water bodies selected for the study

phy, human settlement and water bodies and are shown in Fig. 7. The area of Okhla and Hindon barrage was 27.2 and 28.2 km respectively.

Ground survey was carried out whenever the IRS-1A satellite passed over Delhi from May 1991 to May 1992 to record periodic changes in water bodies, vegetation cover, human settlement and crops. The data were recorded in specialized proforma developed for this study. Relative estimates of larvae were made by dipping method and average density/dip was calculated. Adults were collected during early hours of the day with the help of an aspirator and torch and density was expressed per unit time (MHD). These estimates were made in east, west, centre, north and south directions at fixed catching stations. Larval estimates were made in all water bodies including selected water body at central place and recorded separately to observe the contribution of mosquito breeding by major and minor water bodies.

Multicoloured satellite data of Indian Remote Sensing Satellite (IRS) 1A and B LISS II were obtained from NRSA, Hyderabad in the form of computer compatible tapes. The images were rectified geometrically and radiometrically. Since cloud free data is required for land use classes, further analysis was done only for July, October,

Table 31. Monitoring of land use classes at Okhla barrage using IRS data

Land use classes	Area (sq kms) of land use categories on different dates						
	09.07.1991	05.10.1991	27.10.1991	07.11.1991	12.01.1992	25.02.1992	20.04.1992
Water	4.47 (16.1)	6.56 (23.64)	2.92 (10.51)	3.29 (11.85)	2.37 (8.56)	2.15 (7.75)	2.63 (9.48)
Marshy area	1.12 (4.05)	1.22 (4.40)	2.11 (7.62)	5.81 (20.94)	3.50 (12.22)	3.39 (12.22)	1.30 (4.68)
Weeds	3.8 (13.77)	1.7 (6.36)	3.5 (12.6)	1.58 (5.71)	0.88 (3.17)	3.19 (11.49)	2.73 (9.84)
Plantation	3.97 (14.32)	2.32 (8.36)	3.3 (11.89)	1.28 (4.64)	3.18 (11.47)	2.28 (8.2)	3.06 (11.2)
Agriculture	5.6 (20.29)	4.55 (16.3)	5.21 (18.7)	6.17 (22.2)	6.17 (22.23)	4.67 (16.8)	9.29 (33.47)
Settlement	4.4 (16.18)	9.24 (33.2)	8.25 (29.7)	6.22 (22.42)	5.17 (18.6)	4.11 (14.82)	3.48 (12.53)
Barren land	2.4 (8.66)	1.32 (4.78)	0.88 (3.17)	1.65 (5.94)	5.3 (19.1)	5.64 (20.32)	4.19 (15.11)
Unclassified	1.8 (6.62)	0.75 (2.47)	1.55 (5.6)	1.73 (6.26)	1.08 (3.89)	2.3 (8.29)	1.11 (4.03)

PS – Total area: 27.2 sq km; Figures in parentheses indicate the percentage of the total area

November 1991 and January, February and April 1992 as these images were found free of clouds. Image to image rectification and classification was done with the help of ground data at Vax 11/780 computer system and correlation factor with larval density (D/D) and man hour density (MHD) of mosquitoes with different land use class was calculated by statistical analysis after pooling results from five catching sites located at central, north, south, east and west directions. Similarly, results of minor water bodies prevalent in these sites and supporting the substantial breeding of vector species were also pooled together for statistical analysis.

Results of classified land use class revealed varying degree of changes on each date and class. Okhla barrage constitutes 33.92% types of various water bodies, 14.32% plantation, 20.29% agriculture, 16.18% settlement, 8.67% barrier and 0.62% unclassified in the month of July 1991. The water area was subsequently reduced to 9.48, 8.53, 10.68 and 6.75 in two dates of October, November, January, February and April respectively. Similarly, variation in other classes was also observed at different time interval. Plantation area varied from 14.32 to 4.64% agriculture land 16.3 to 33.47%, settlement class 14.2 to 33.2%, barren land 0.88 to 5.64% and unclassified category from 0.75 to 8.29% (Table 31 and Fig. 8).

Hindon barrage constituted 11.16% types of different water bodies, 10.16% plantation, 13.19% agriculture, 31.75% settlement, 24.3% barren land and 9.3% unclassified land. Variation at different time intervals were 18.74 to 5.1% water bodies, 19.42 to 0.521% plantation, 31.96 to 13.19% agriculture, 28.15 to 42.5% settlement, 36.4 to 2.65% barren land and 01.46 to 9.3% in unclassified category (Table 32 and Fig. 9).

Results of immature and adult densities of mosquitoes of all subsites were pooled together in Okhla and Hindon barrage and are presented in Table 33. The density of mosquitoes starts building up from July onwards and attains its peak (278.8) in October followed by gradual decline in winter months and showed a peak again in the month of April (314.2) in Okhla barrage. Similar trend was observed with the larval density. Highest larval density (321.6) was recorded in October and April (175.0) which indicates a good correlation of larval and adult densities. In Hindon barrage the density of mosquitoes remained quite high from October to April (126 to 313) with annual high densities even during winter months. This may be due to the fact that the human settlement is quite thick in Hindon area as compared to Okhla barrage. Larval density was also found consis-

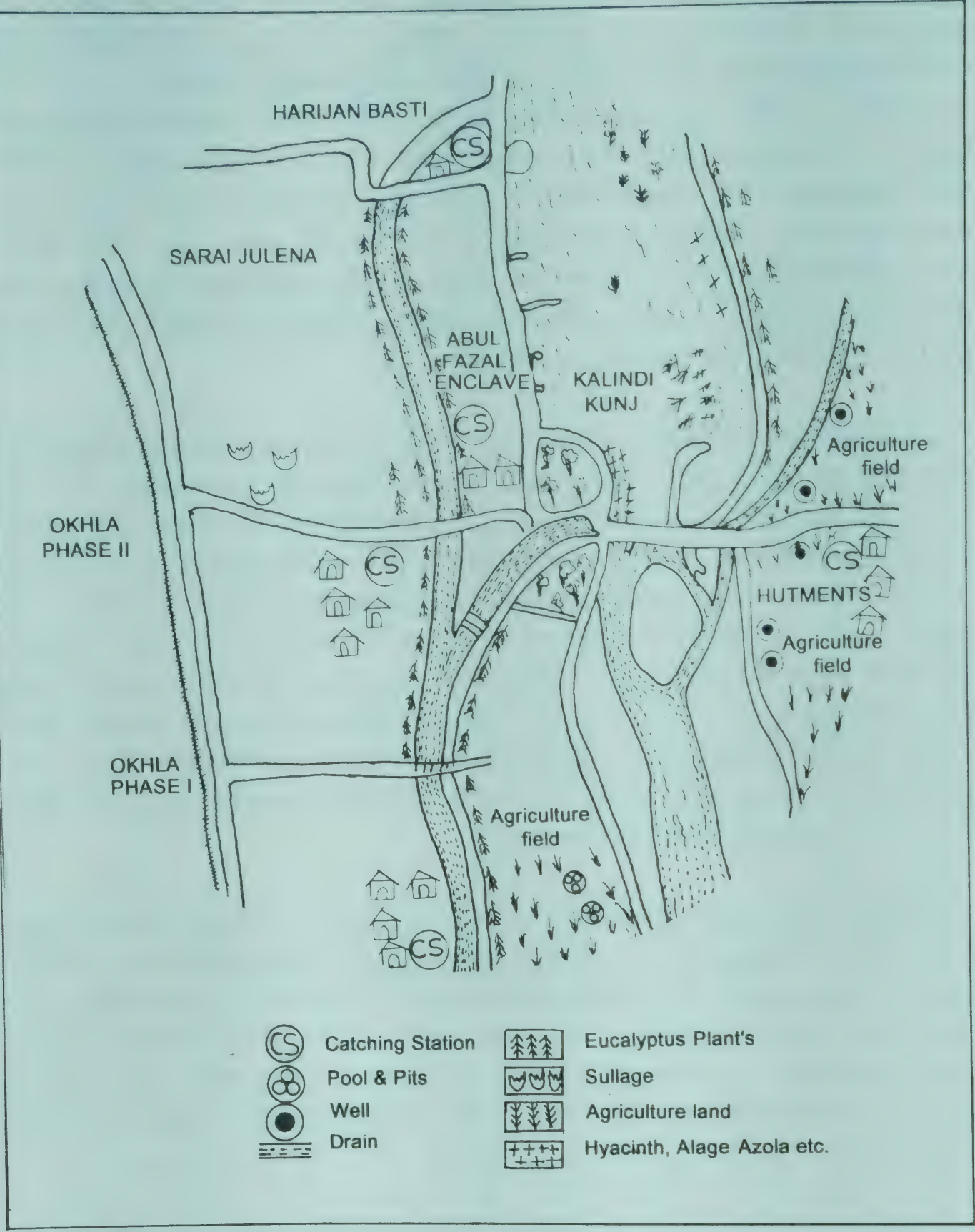


Fig. 8: Land use classes in Okhla Barrage

tently high except in the month of February and April where 15.0 and 26.84 per dip density was recorded.

Table 34 depicts the correlation factor values in respect of land use classes and relative densities of immature and adult mosquitoes in Okhla and Hindon barrage. Correlation factor in respect of settlement, barren and unclassified land category was not attempted. Results of statistical analysis revealed that both negative and positive

Table 32. Monitoring of land use classes at Hindon barrage using IRS data

Land use classes	Area (sq kms) of land use categories on different dates						
	09.07.1991	05.10.1991	27.10.1991	07.11.1991	12.01.1992	25.02.1992	20.04.1992
Water	0.52 (1.86)	0.86 (3.05)	1.88 (3.84)	1.12 (3.97)	1.20 (4.26)	0.91 (3.24)	0.74 (2.63)
Marshy area	2.64 (9.30)	4.43 (15.69)	2.93 (10.40)	0.20 (0.73)	1.08 (3.83)	1.75 (6.22)	1.01 (3.60)
Weeds	- -	- -	0.01 (0.038)	0.10 (0.4)	0.28 (1.02)	0.86 (3.07)	0.26 (0.94)
Plantation	2.87 (10.16)	2.09 (7.43)	4.26 (15.1)	5.48 (19.42)	4.84 (17.17)	2.16 (7.65)	1.48 (5.21)
Agriculture	3.72 (13.19)	8.24 (29.21)	5.7 (20.19)	4.92 (17.44)	7.6 (26.92)	9.02 (31.96)	3.93 (13.92)
Settlement	8.96 (31.75)	7.94 (28.15)	10.9 (38.71)	9.36 (33.16)	12.01 (42.5)	9.84 (34.89)	9.88 (35.01)
Barren land	6.86 (24.3)	3.47 (12.29)	2.83 (10.04)	6.44 (22.94)	0.74 (2.65)	3.01 (10.68)	10.27 (36.4)
Unclassified	2.63 (9.3)	1.17 (4.15)	0.4 (1.64)	0.53 (1.84)	0.41 (1.46)	0.63 (2.25)	0.61 (2.16)

PS: Total area : 28.2 sq km; Figures in parentheses indicate the percentage of the total area

Table 33. Relative densities of immatures and adults in and aruond Okhla and Hindon barrage

Monitoring dates	Okhla Barrage						Hindon Barrage					
	MHD			D/D			MHD			D/D		
	Culex	*Anopheles*	Total	*Culex*	*Anopheles*	Total	*Culex*	*Anopheles*	Total	*Culex*	*Anopheles*	Total
9.7.91	11.8	4.4	16.2	22.6	8.4	31.0	43.0	0.7	43.7	30.5	1.3	31.8
5.10.91	33.6	57.6	91.2	28.0	47.8	75.8	103.8	13.0	126.8	92.44	8.94	101.38
27.10.91	251.0	27.8	278.8	313.2	28.4	321.6	202.6	12.8	215.4	84.88	6.7	90.95
7.11.91	119.4	18.6	218.0	231.6	20.0	251.6	268.4	19.2	287.6	50.12	3.2	53.14
12.1.92	30.0	45.6	75.6	19.2	35.4	54.6	238.4	17.0	255.4	82.84	0.4	83.24
25.2.92	107.6	8.8	116.4	38.0	19.8	57.8	250.8	8.6	259.4	14.06	0.4	15.0
20.4.92	309.0	5.2	314.2	165.0	10.0	175.0	305.0	8.6	313.6	23.94	2.9	26.84

MHD – Average man hour density; D/D – Average larval density/dip

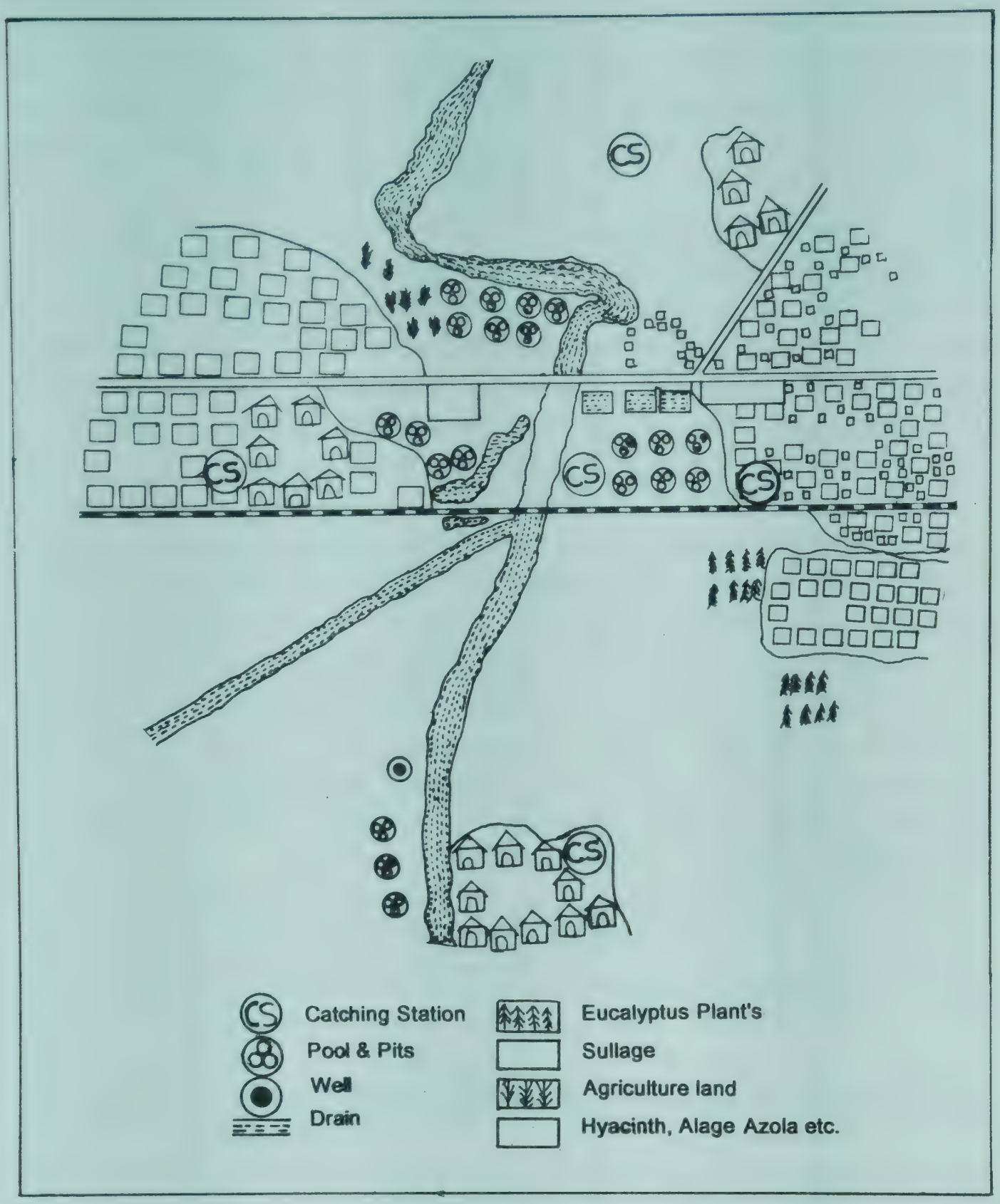


Fig. 9: Land use classes in Hindon Barrage

correlation was found with different sites and land use classes. In Okhla barrage a negative correlation (r = –0.45) was observed in respect to water bodies against man hour density of *Culex* mosquito as against positive correlation (r = 0.54 and 0.54) in Hindon barrage in respect of adult and larval density of *Culex quinquefasciatus* species and also with total mosquitoes (r = 0.59 and 0.51). Similarly, a positive correlation (r=0.45 and 0.69) was also observed in respect of marshy land use class and immature density of culicine and anopheline in Hindon barrage only. A positive and negative correlation (r =0.58 and –0.66) in respect of weeds, anopheline immature and adult den-

Table 34. Correlation factor(r) values in respect of land use classes and relative densities of immature and adult mosquitoes in Okhla and Hindon Barrage

Land use classes	Okhla						Hindon					
	Culex		*Anopheles*		Total mosquitoes		*Culex*		*Anopheles*		Total mosquitoes	
	MHD	D/D	MHD	D/D	MHD	D/D	MHD	D/D	MHD	D/D	MHD	D/D
Water	-0.445*	-0.29	0.48**	0.46**	-0.39	-0.24	0.54**	0.54**	0.91**	0.05	0.59**	0.51**
Marshy	0.09	0.24	-0.0114	1.0109	$9.88x10^{-2}$	0.27	-0.74	0.45**	-0.32	0.69**	-0.72*	0.49**
Weeds	0.20	0.18	-0.66*	0.58*	$9.18x10^{-2}$	0.09	0.17	-0.69*	-0.54*	-0.73*	0.108	-0.72
Plantation	-0.85*	-0.20	-0.16	0.24	-0.226	-0.26	0.16	0.43	0.66**	-0.109	0.19	0.39
Agriculture	0.60	0.23	-0.14	-0.52*	0.568**	0.195	0.05	0.294	0.32	0.08	0.08	0.29

PS : *Indicates negative correlation; **Indicates positive correlation

sity was also observed in Okhla barrage as against negative correlation (r = –0.54 and –0.73) in Hindon barrage. There was also a positive correlation (r = 0.6) in respect of agriculture and *Culex* adult density in Okhla barrage.

The study revealed both positive and negative correlation in different water bodies. In Okhla barrage water constituted 33.92% area in the month of July as such breeding was washed away and it started building up when water recorded and created numerous small water bodies which were conducive for mosquito breeding. This was the reason that a negative correlation was found in this site. Contrary to this, in Hindon barrage the water bodies constituted only 10.16% area and mosquito breeding started only when more water bodies were created in subsequent months. This may be the reason for positive correlation observed in this site. This indicates that remote sensing technique is quite sensitive to map qualitative and quantitative changes in large sized water bodies. However, minor water bodies which contribute maximum breeding are not covered with present resolution (72 x 72 m) but may be delineated in future with further advancement of satellite sensors. Another limitation is the cloud cover particularly in monsoon period which coincides with the transmission period of malaria. Therefore, this technique may not be appropriate in detecting the breeding potential of *An. culicifacies*, principal vector of rural malaria which breed in smaller water bodies or in captivity but may be appropriate for *Culex quinquefasciatus* or other *Culex* species which breed generally in large water bodies.

A Feasibility Study using Remote Sensing Technique in the Estimation of Larval Production

Mosquitoes are associated with a variety of aquatic habitats which are species specific. Larval breeding surveys are time consuming, costly and cumbersome. Therefore, a feasibility study to use remote sensing images in assessing mosquitogenic potential was carried out. Sanjay lake and Chintamani lake with their surrounding area within a radius of 3 km in east of Delhi were selected for study. Images of the same region were obtained from Indian Remote Sensing Satellite (IRS) IA and B.

The satellite IRS passes over Delhi after every 21 day interval at 1100 h. During the study period Regional Remote Sensing Services Centre (RRSSC) could receive data only for 7 dates. The images were rectified geometrically and radiometrically by RRSSC, Ban-

galore. The images were analyzed on 512 x 512 pixel frame and each pixel corresponds to 30 x 30 meter. The Sanjay lake shoreline was divided into 50 segments each of 30 m to match with the size of one pixel (Fig 10). Ground truth information was collected from each segment on every satellite pass. From each segment mosquito larval density were recorded. Adults were also collected from human dwellings and cattlesheds situated nearby and man hour density was calculated.

Besides, the data collected from Sanjay lake, larvae and adults were also collected from its surrounding area within 3 km through-

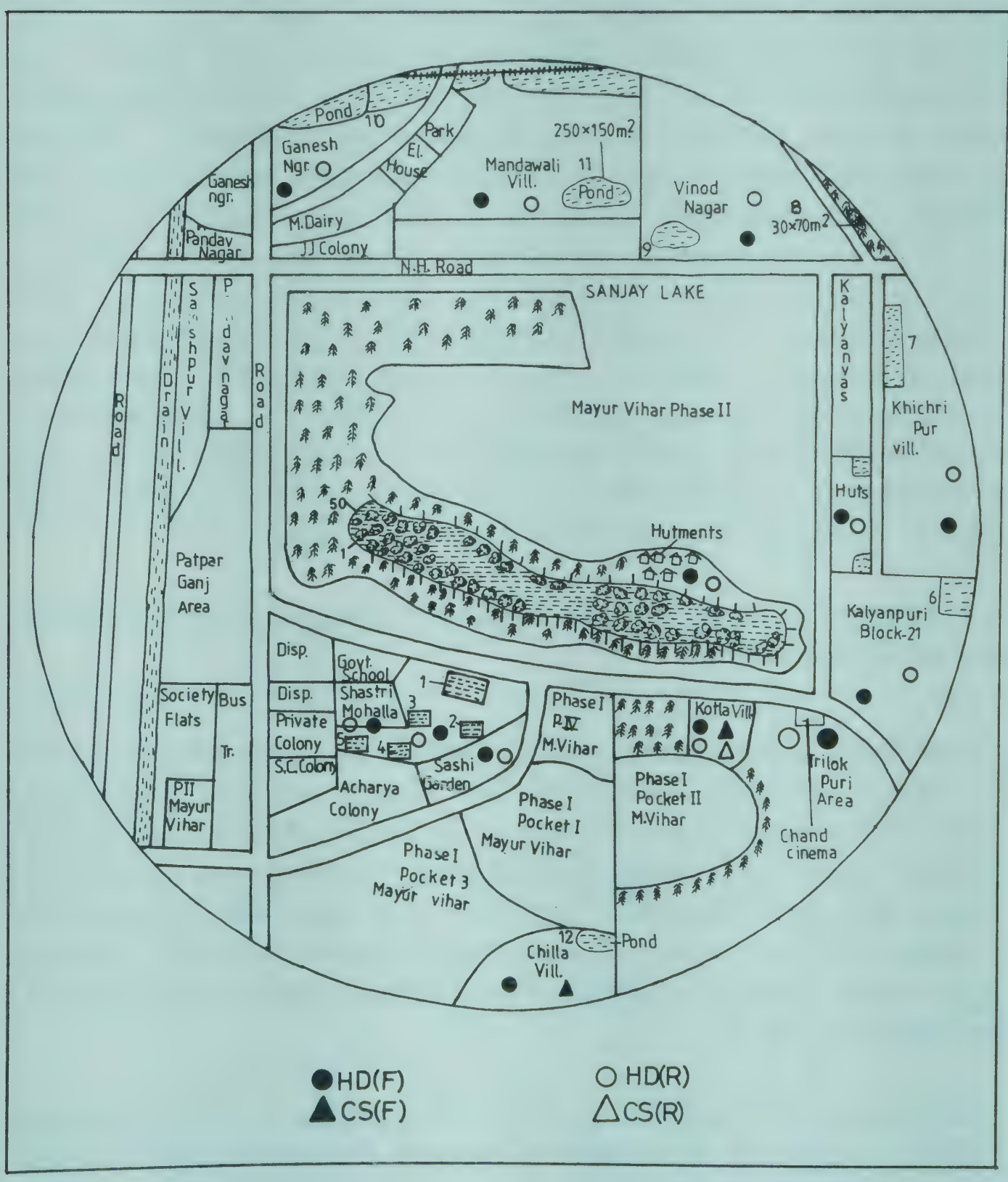


Fig. 10: **Sanjay lake showing its surrounding areas**

out the study period. Ground information has also been recorded to monitor temporal changes in water bodies, vegetations, agriculture cropping etc.

To find out the mosquito production per sq m by the lake and its surrounding 12 ponds a multiplication factor was calculated. It was assumed that while taking a dip, the dipper moves a distance of 0.3 meter, and since 'r' is the radius of the dipper '2r' is the width of the movement area,

$$\text{Actual area covered per dip} = 2r(0.3) + \pi r^2$$

$$= r\,(0.6 + \pi r) = 0.0378 \text{ sq m}$$

The larvae/dip is actually larvae per 0.0378 (sq m). Hence larvae per sq m = 1 x 0.0378

= 26.5 (Multiplication factor (MF) for larval production)

The total 27.7 km land areas have been classified into 7 main classes : water, weed, plantation, agriculture vegetation, agriculture fallow barren, urban and barren. The percentage water area varies from season-to-season shown in the Table 35. The maximum percentage of water area was recorded during the month of February 1992 (3.7%) followed by November 1991 (3.5%) and October 1991 (3.25%). This water area was further classified into 2 sub-classes viz. lake water and surrounding water. The maximum area covered by lake water i.e. 26,1900 sq m was during July 1991 and minimum 14,6700 sq m in October 1991.

The larval density per dip of Sanjay lake varies from 0.13 (February 1992) to 13.54 (November 1991). The number of pixel classified as water in lake and the larval density showed a poor negative correlation (r = –0.233) but the negative sign shows that increase in water in the lake results in decrease in the larval density (Table 36).

Table 37 gives the total larval production from the Sanjay lake on different dates. Maximum mosquito production was also observed in the month of October 91 and minimum in the month of February 92. The co-relation of the breeding area with larval production is positive 0.445 (Q). It means when area of breeding increases the larval production also increases. Total larval production and man hour density of adult mosquitoes also showed significant correlation with density/dip (r = 0.55 and 0.48 respectively).

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Table 35. Estimated area of land use classes at Sanjay lake site using IRS images

Land use classes	Area (sq km) of land use categories on different dates						
	9.7.1991	5.10.1991	27.10.1991	7.11.1991	12.1.1992	25.2.1992	20.4.1992
Water	0.864	0.910	0.383	0.974	0.504	1.033	0.453
	3.119	3.285	1.384	3.516	1.819	3.730	1.634
Weed	0.316	1.985	0.616	0.764	0.260	0.157	0.595
	1.141	7.166	2.222	2.758	0.939	0.565	2.148
Plantation	5.756	1.618	1.671	1.616	1.048	1.922	1.994
	20.780	5.841	6.034	5.832	3.782	6.940	7.197
Ag. veg.	1.514	4.192	3.389	4.326	1.881	2.308	3.326
	5.466	15.134	12.236	15.618	6.791	8.331	12.005
Ag Fallow barren	1.467	4.704	4.462	4.093	7.287	2.558	4.425
	5.296	16.982	16.109	14.777	26.308	9.234	15.976
Urban	11.414	8.778	10.032	7.571	10.120	9.788	11.086
	41.206	31.690	36.218	27.331	36.533	35.337	40.022
Barren	3.646	3.216	5.625	7.588	5.671	8.090	5.635
	13.162	11.609	20.307	27.393	20.473	29.206	20.343

PS: Total area: 27.7 sq km; Lower figures indicate the % of the total area

Table 36. Larval density on different dates in Sanjay lake

Date	Total water area in lake X^1	Total no. of larvae	D/D X^2
9 Jul 91	261900	289	1.156
5 Oct 91	171900	1143	4.572
27 Oct 91	146700	151	0.604
7 Nov 91	195300	3385	13.54
12 Jan 92	208800	219	0.876
25 Feb 92	234900	34	0.136
20 Apr 92	224100	74	0.296

Total no. of segment = 50; Total no. each date = 250 (one dip in 6 m); r (X^1, X^2) = – 0.233 where X^1 – > Total water area in lake; X^2 – > Density/Dip.

Table 37. Total breeding area and larvae production on different dates in Sanjay lake

Dates	Total breeding area X^1	D/D	Total larvae X^2	Total larvae production/ sq m
Jul 91	889.5	1.2	28286.10	31.8
5 Oct 91	3276.9	4.6	399454.11	121.9
27 Oct 91	2740.2	0.6	43569.18	15.9
7 Nov 91	938.6	13.5	335784.15	357.75
12 Jan 92	750.0	0.9	17887.5	23.85
25 Feb 92	1457.6	0.1	3862.64	2.65
Apr 20 92	1159.5	0.3	9218.03	7.95

r(X^1, X^2) = 0.445

Besides the total larval production of the Sanjay lake, the total production for the 12 surrounding remote sensing identifiable ponds was also estimated. Total larval production of Sanjay lake and its surrounding ponds reveal that ponds produced higher number of larvae per sq m during the month of October, 1991, January, February and April, 1992. The prodution in Sanjay lake was more during October

Table 38. Larvae production per sq m in 12 surrounding ponds and Sanjay lake

Date	Larvae production/sq m in	
	12 ponds	Sanjay lake
5 Oct 91	72.1	121.9
27 Oct 91	27.1	15.9
7 Nov 91	36.3	357.75
12 Jan 92	34.2	23.85
25 Feb 92	12.5	2.65
20 Apr 92	20.9	7.95

and November, 1991 as compared to ponds because of some environmental factors (Table 38).

Modelling of Terrain-based Probability of Mosquitogenic Conditions using Geographic Information System (GIS) Approach: A Pilot Study in Car Nicobar Island

A collaborative study between Malaria Research Centre, Delhi and Indian Institute of Remote Sensing (IIRS), (Department of Space), Dehradun was started to find out the application of Geographic Information System (GIS) approach for modelling terrain-based probability of mosquitogenic conditions.

Data on Car Nicobar in respect of vegetation, water bodies, number of wells, location of human settlements and annual parasite index (API) in connection with malaria, were collected and overlays have been developed. The simulated 3D view of the Island, and perspective of particular areas (Figs. 11 and 12) were developed based on the survey of India toposheet. The digital terrain (DTM) model was also developed.

Satellite data of Car Nicobar (February 1992) have also been analzsed partially and various overlays will be superimposed over DTM.

Preliminary results indicate that by digital terrain model, we can predict the possible water logging zones. As in case of Car Nicobar Island the possible water logging/mosquito breeding zone is on the pe-

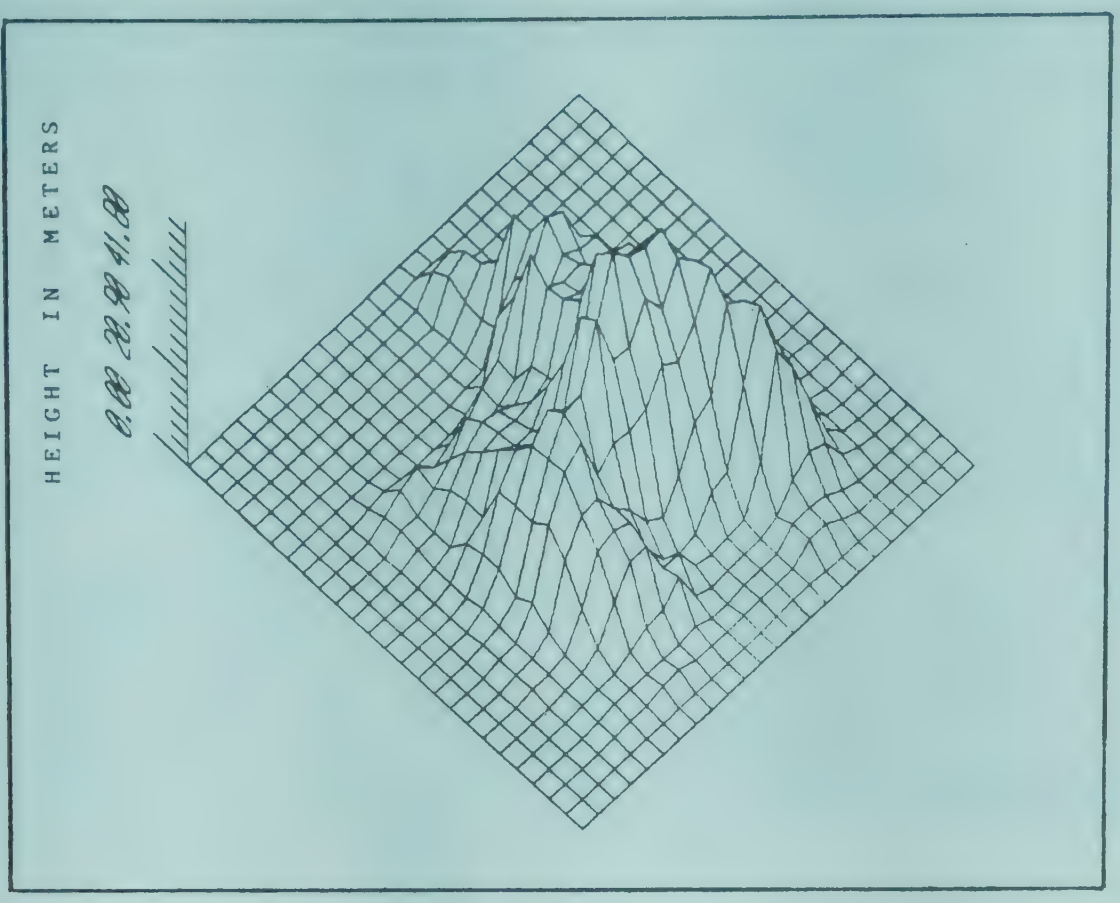


Fig. 11: Simulated 3-D view of Car Nicobar Island

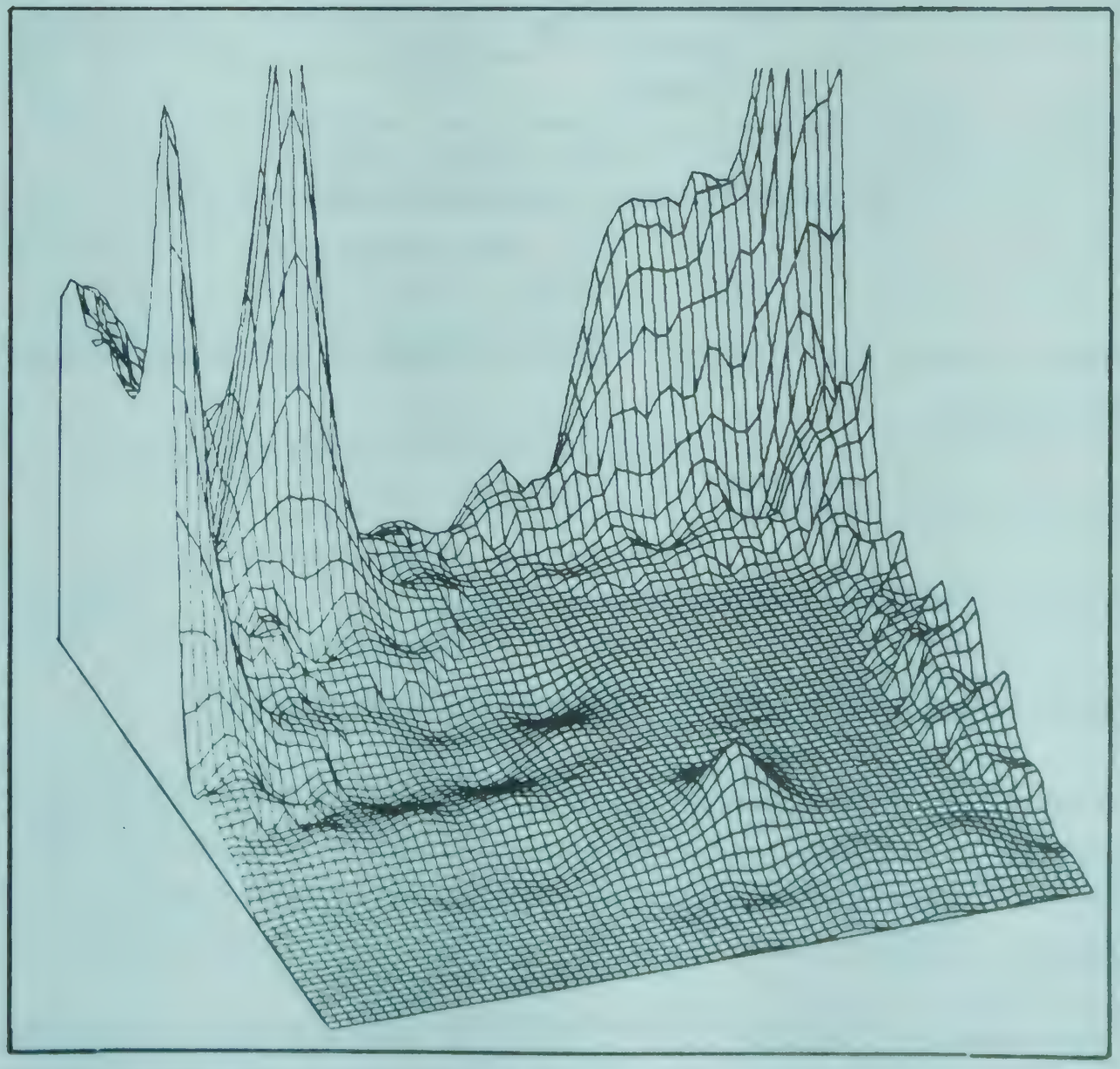


Fig 12: Perspective of Kimios area (NNE looking)

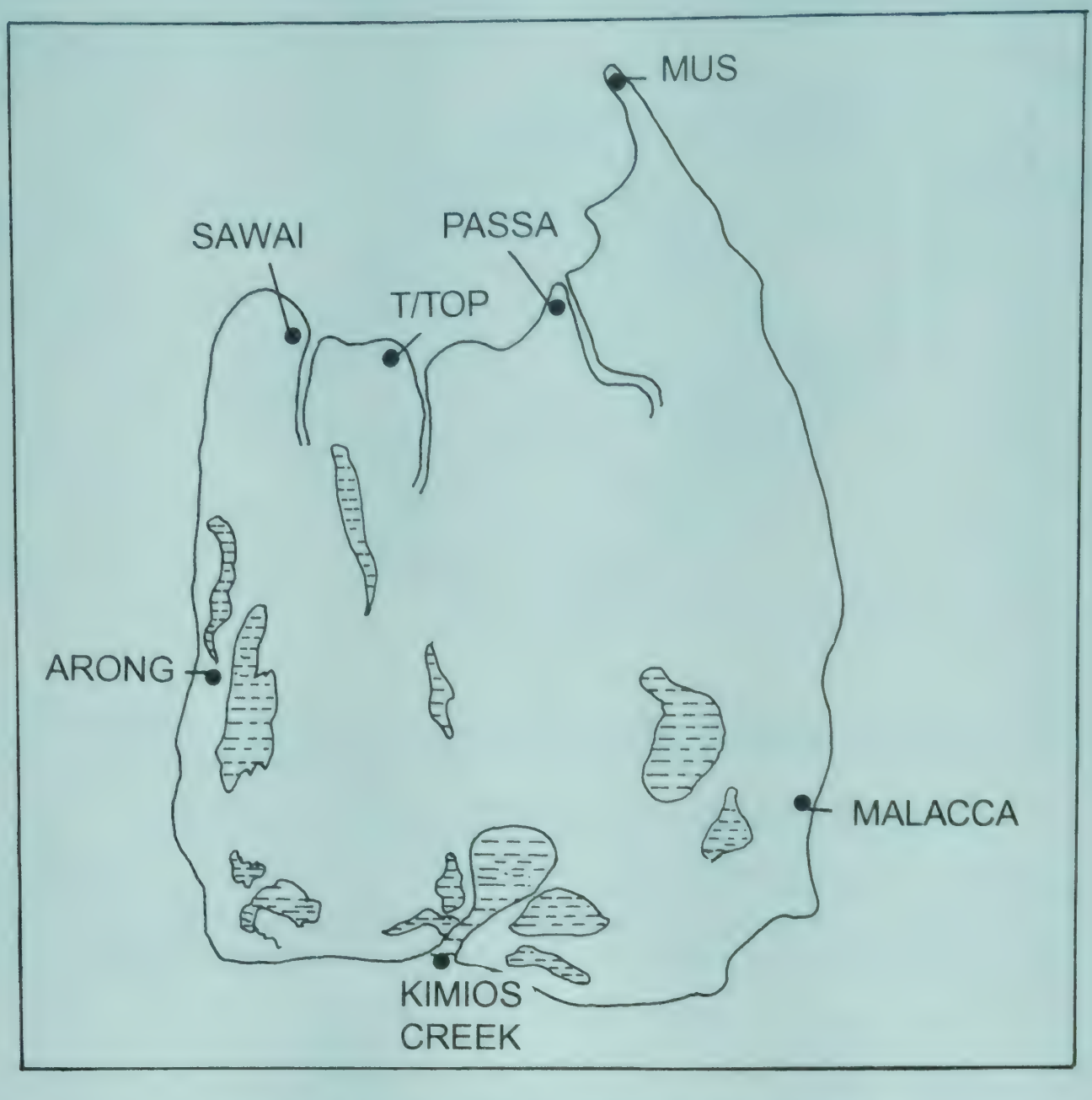


Fig. 13: Major water bodies in Car Nicobar

riphery of the Island (Fig. 13) because terrain being flat is prone to water logging.

The study is in progress.

Studies on Sandfly Vectors

Field Evaluation of Neem Oil as Repellent to *P. papatasi*

Laboratory studies have proved that neem oil acts as a good repellent to *P. papatasi*. Therefore, a field study was carried out in Chaiyan village of Sri Ganganagar district in Rajasthan which is endemic for oriental sore.

Four tests i.e. heating of 5% neem oil mat (cardboard mat measuring 35 mm x 25 mm x 3mm soaked in 1 ml of 5% neem oil in

Preparing rearing cups for evaluation of biolarvicide against sandfly larvae

acetone) on an electric heater, topical application of 2% neem oil (in mustard oil), mustard oil and control (without any oil) were carried out for 5 consecutive nights.

It was found that topical application of 2% neem oil provided 97.6% protection for 10 h. On volunteers with mustard oil landing of sandflies commenced after 2400 h and it provided 91.8% protection. On volunteers with 5% cardboard neem mat burnt in their room, not a single sandfly landed (Table 39).

Table 39. Results of evaluation of neem oil preparation as repellent to *Phlebotomus papatasi*

Sandflies	Mean no. female sandflies alighted/caught			
	5% neem mat	2% neem oil	mustard oil	Control
Alighting sandflies	0.0 (0)	0.4 ± 0.27 (2)	1.4 ± 0.27 (7)	17.2 ± 2.58 (86)
Total resting sandflies caught	9.6 ± 2.79 (48)	25.6 ± 2.79 (128)	32.2 ± 5.72 (161)	33.0 ± 5.17 (165)

Figures are in mean ± S.E.; Figures in parentheses denote the number of sandflies alighted/caught

The results indicate that topical application of 2% neem oil may provide 100% protection against *P. papatasi* if applied at 2100 h. 5% neem mats can also be used as effective space repellent and a substitute for chemical pyrethroids for use on electric heaters.

Similar field studies on *P. argentipes* in endemic area of Kala-azar in Bihar are being carried out.

Effect of Minor Engineering Intervention in Natural Breeding Habitats of Sandflies

A minor engineering intervention in mud houses/cattlesheds by constructing a brick made cement plastered skirting of 9" on wall (vertical) and 9" on floor (horizontal) was done in 7 mud houses/ cattlesheds in Sirora village for reducing/eliminating the breeding grounds of sandflies (*P. papatasi*). Four houses without intervention served as control. The density of sandflies was monitored before and after the intervention from October 1991 to March 1993.

The analysis of results (Fig. 14) revealed that by minor engineering, there was a remarkable reduction in the density of *P. papatasi*

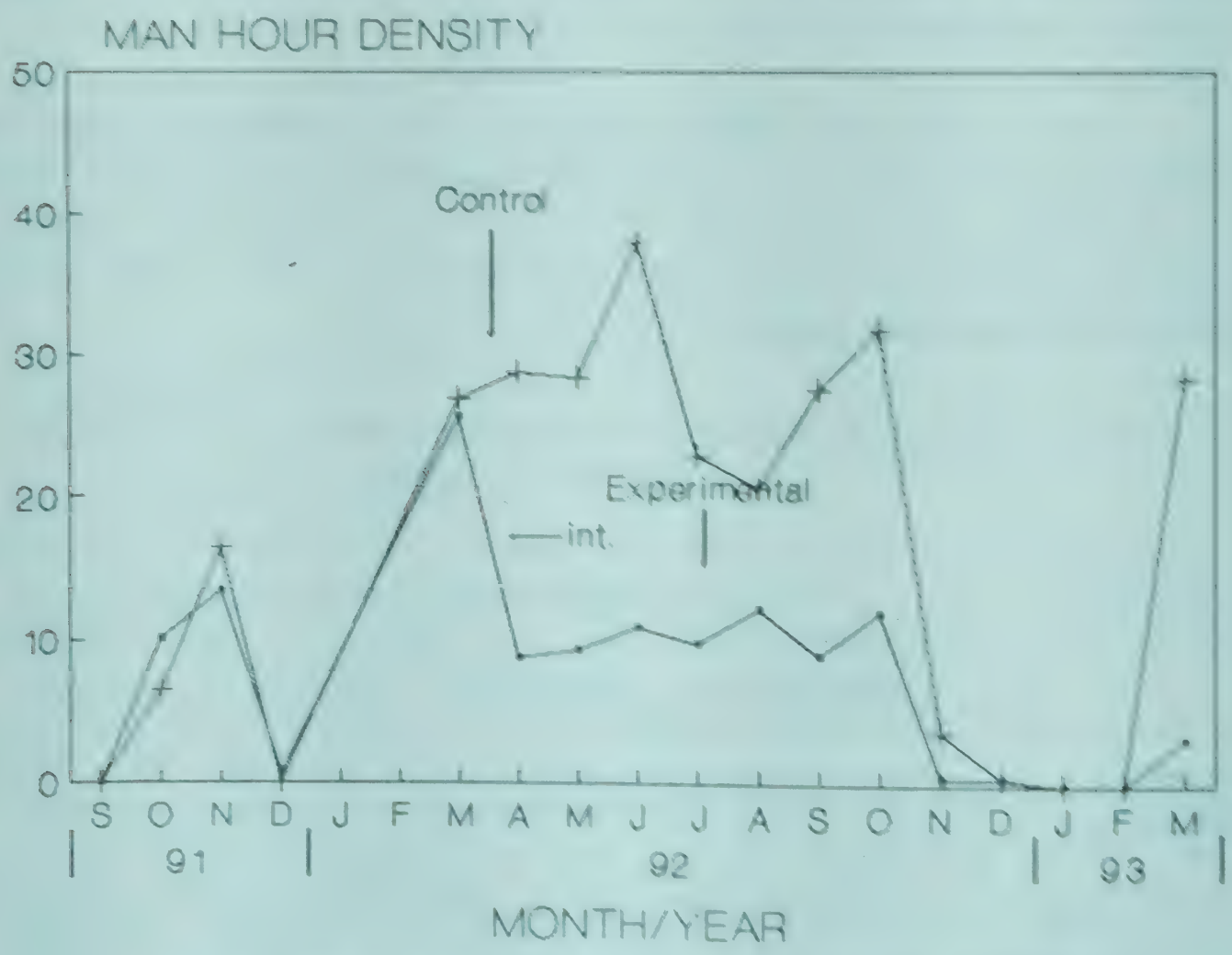


Fig. 14: Effect of minor engineering intervention (in mud houses) on density of *P. papatasi*

Table 40. Toxicity of bioinsecticides against the larvae of *P. argentipes*

Bioinsecticide & dose	Stage of larvae	% mortality on day									
		1	2	3	4	5	6	7	8	9	10
B. sphaericus		74.0	76	84	84.0	88.0	100				
0.5%	Ist	(0)	(0)	(0)	(0)	(0)	(0)				
	IIIrd	4	12	24	46	48	54	68	84	100	
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
Bactoculicide											
0.5%	Ist	100									
		(0)									
	IInd	80	92.0	100							
		(0)	(0)	(0)							
	IIIrd*	10	26.6	43.3	73.3	93.3	100				
		(0)	(0)	(0)	(0)	(0)	(0)				

*3 replicates; Figures in parentheses are % mortality in control

as compared to control. However, total reduction could not be achieved. The reason for the presence of sandflies in some experimental houses may be attributed to the presence of many holes/cracks in dilapidated walls of cattlesheds above the level of skirting which could have served as breeding ground for sandflies.

It therefore seems logical to consider the filling up of indentations in wall above skirting level and extension of intervention in all sandfly potential houses simultaneously.

Evaluation of Biolarvicides against *Phlebotomus argentipes*

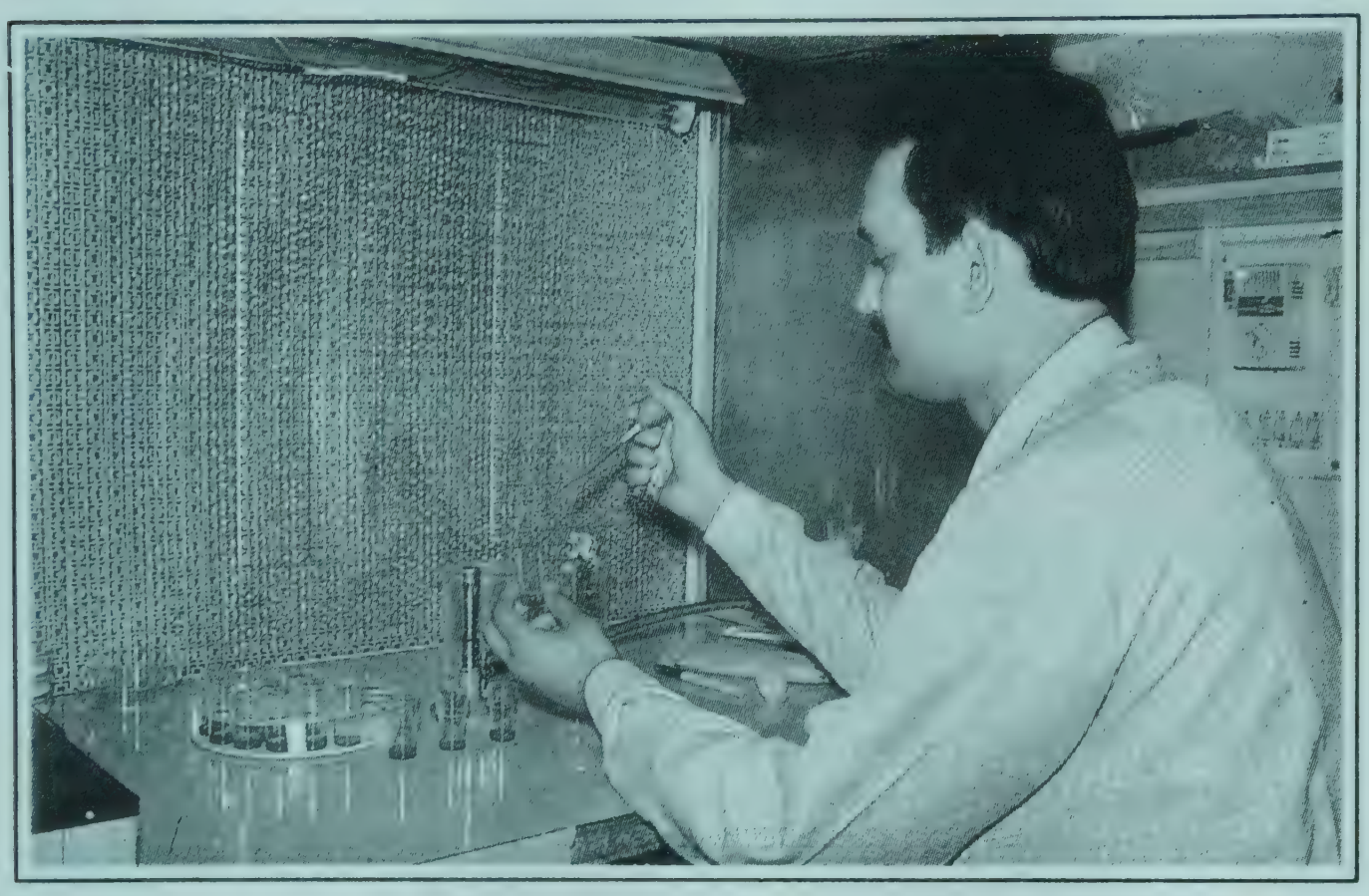
Both 'Bactoculicide' (*Bacillus thuringiensis* var. israelensis H14) and 'Spherix' (*Bacillus sphaericus* B101) against larvae of *P. papatasi* have shown 100% mortality of sandflies larvae within 5 to 7 days. Therefore, the toxicity of these biolarvicides was evaluated against *P. argentipes*, the vector of Indian Kala-azar.

Dust formulation 0.5% of both biolarvicides were used individually by mixing in sandfly larval food in rearing cups. Ten unfed larvae were released in each rearing cup. The control cups contained unfed larvae and normal sandfly larval food. The daily mortality was recorded by observing the cups under stereomicroscope. Five replicates with various stages of larvae were set up unless otherwise stated. The results (Table 40) revealed that with Bactoculicide, III stage larvae died within 6 days while with Spherix, 100% mortality of larvae was achieved in 9 days. There was inverse instar susceptibility relationship.

The feasibility of application of biolarvicides against sandflies under field condition is being studied.

Malaria Parasite Bank

Malaria parasite bank is a centralized facility to cater to the needs of scientists and research scholars working on various aspects of malaria. Human and non-human plasmodial species and strains collected from different geographical regions are being maintained *in vivo*, *in vitro* and under cryopreserved conditions.



Cultivation of malaria parasites *in vitro*

Human Malaria Parasites

P. falciparum isolates collected from field (Table 41) were adapted to *in vitro* cultivation in RPMI-1640-HEPES medium supplemented with 10-15% human AB+ serum and A+ erythrocytes following the candle jar technique of Trager and Jenson (1976). The candle jar can be replaced by CO_2 incubator. Out of a total of one hundred eighteen isolates collected, 93 got adapted in culture and were cryopreserved. All the adapted *P. falciparum* isolates were subjected to periodical revival and maintenance *in vitro*. An infective gametocyte producing *P. falciparum* strain NF 54 from CDC Atlanta, USA

Table 41. Details of *P. falciparum* isolates collected and adapted *in vitro*

Areas	Total	Adapted/Cryopreserved
Delhi	46	37
Ghaziabad	27	22
Shankargarh	9	8
Shahjahanpur	6	6
Mandla	23	15
Sonapur	2	2
Rourkela	4	2
Rameshwaram	1	1
Total	118	93

and 3D7A, a clone of NF54 from London School of Hygiene and Tropical Medicine, London are also being maintained in the bank.

Cloning of *P. falciparum* Isolates

It is known by earlier studies that isolates of *P. falciparum* are often not genetically homogenous. This has been shown by variation in drug susceptibility, isoenzymes, changes in certain characteristics at protein level and in antigens. Therefore, by establishing clones from such heterogenous population of parasites, a precise analysis of the genetic composition of the parasite isolates can be made.

Five isolates of *P. falciparum* collected from various parts of the country were grown and adapted *in vitro*. These isolates showed a variable grades of chloroquine susceptibility. All these isolates were tested for isoenzymes like GPI, GDH and LDH. They were found to be homogenous for these enzymes. All five isolates were cloned by limiting dilution method in 96 well flat bottom tissue culture plates. Clones thus obtained were tested for chloroquine (CQ) susceptibility, seroreactivity and stability in culture after several passages (Table 42).

Results show that these isolates are heterogenous in regard to their response to chloroquine *in vitro*. The CQ sensitive isolates contain a proportion of resistant parasites which may remain unnoticed in *in*

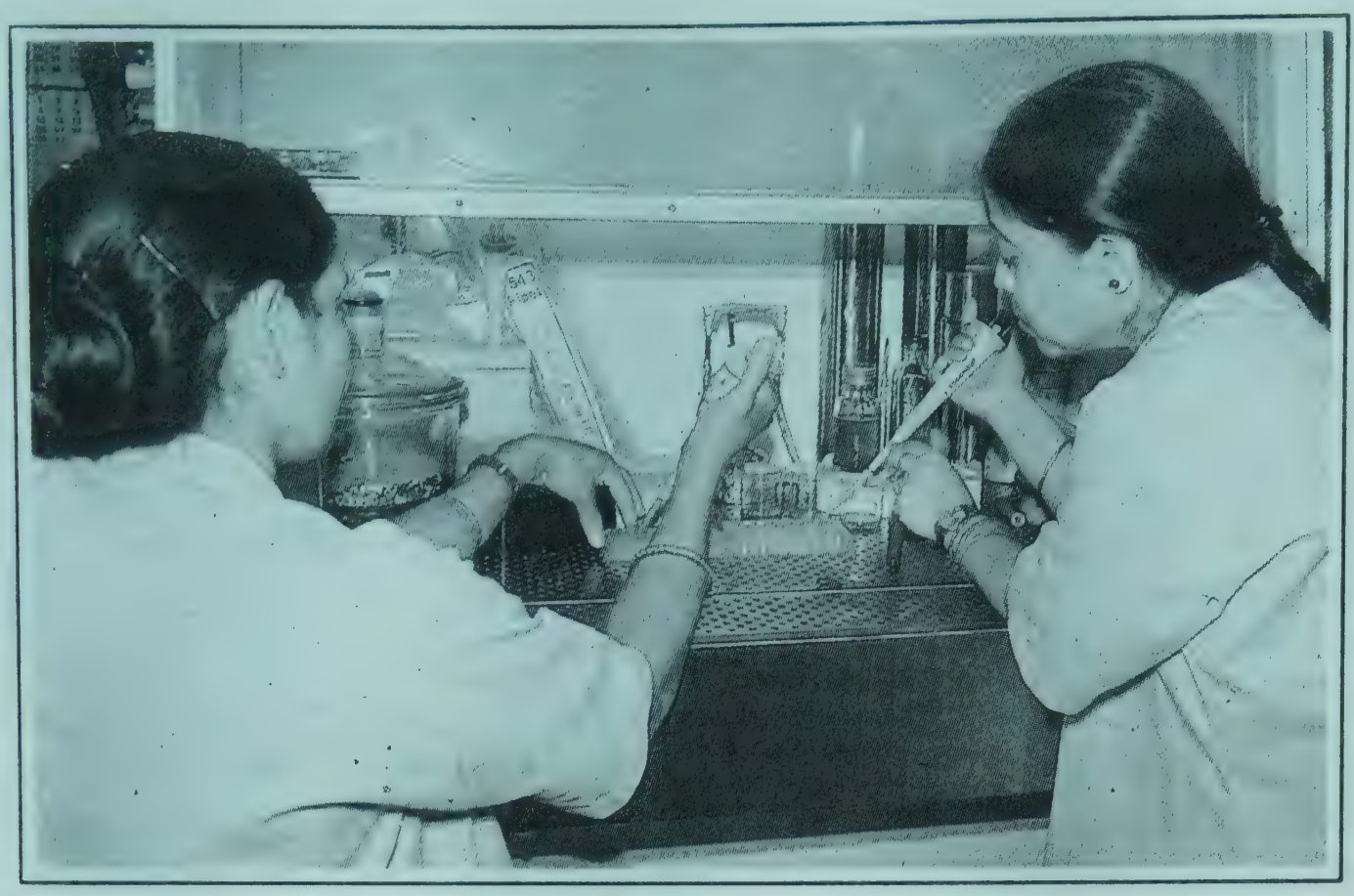
Table 42. Characterization of isolates and their clones

	FSJ-A6 (Shahjahanpur)	FJB-D2 (Jabalpur)	FJB-D4 (Jabalpur)	Sh-4 (Shankargarh)	FDL-1 (Delhi)
Chloroquine sensitivity					
In vivo	S	S	R	R	S
In vitro					
IC_{50}	1.2 pm (0.24×10^{-6} M/L)	1.3 pm (0.26×10^{-6} M/L)	6.75 pm (1.35×10^{-6} M/L)	2.5 pm (0.5×10^{-6} M/L)	2.85 pm (0.57×10^{-6} M/L)
IC_{90}	2 pm (0.4×10^{-6} M/L)	3.5 pm (0.7×10^{-6} M/L)	17.5 pm (3.5×10^{-6} M/L)	14.5 pm (2.9×10^{-6} M/L)	4.7 pm (0.94×10^{-6} M/L)
Growth rate	4.92 $\pm$ 0.2	4.1$\pm$0.25	8.48$\pm$0.24	10.57$\pm$0.45	5.53$\pm$0.41
Seroreactivity by IFA test	1mg	1mg	10 mg	5 mg	0.1 mg
No. of stable clones obtained	15	16	16	8	7

contd...

Table 42. (*contd.*)

	FSJ-A6 (Shahjahanpur)	FJB-D2 (Jabalpur)	FJB-D4 (Jabalpur)	Sh-4 (Shankargarh)	FDL-1 (Delhi)
No. of clones formed gametocyte	1	4	1	3	3
Chloroquine susceptibility in clones					
S	15	14	12	7	6
R	0	2	4	1	1
(MIC)		6pmol	8-20 pmol	10 pmol	8.5 pmol
		or 1.2×10^{-6} M/L	or $1.6\text{-}4 \times 10^{-6}$ M/L	or 2×10^{-6} M/L	or 1.7×10^{-6} M/L
Seroreactivity	10 +ve (0.5-30 mg)	S 11+ve (1-50 mg) R 1+ve (100mg)	S 9+ve (2-100 mg) R 3+ve (50-100 mg)	S 7+ve (10-100 mg) R 1-ve	S 6+ve (1-20mg) R 1 +ve (10 mg)



Parasite cloning

vivo monitoring and also in *in vitro* drug testing in uncloned materials. Variation in the growth rates of these isolates has been noticed, therefore, changes may occur in some clones during long period of culture by overproducing. Regarding the seroreactivity, parasites from all the sets have shown positivity with human immune IgG, only some clones demonstrated positivity at low concentration of antibody like uncloned parasites.

Sudies on the Genetic Variability of Antigen of *Plasmodium falciparum* of Indian Origin

The study is being carried out in collaboration with Dr. V.S. Chauhan, ICGEB, Delhi. The genetic variablity of different antigens of different stages of malaria parasites in susceptible hosts is of great concern towards the development of possible vaccine against malaria. So far no studies have been made on variability of antigen genes of malaria parasite of Indian origin. Genetic variability of these genes with particular emphasis on merozoite surface antigen (MSA) 1 and 2 and thrombospondin related anonymous protein (TRAP) of the Indian isolates are being investigated by polymerase chain reaction (PCR), cloning and sequencing.

Genomic DNA was isolated from Indian isolate of *P. falciparum* FCD3 strain maintained in *in vitro* culture in ICGEB. Primers (23

mer) corresponding to the 5' and 3' terminal end of the MSA2 were synthesized by PCR with denaturation at 94°C for 1 min, annealing at 55°C for 1 min and amplification at 72°C for 3 min for 35 cycle. The PCR amplified product after purification was cloned at the Srf I site (blunt end ligation) into PCR script SK (+) cloning vector (stratagene). Positive clones were selected by both PCR and restriction enzyme (Pvu II) digestion. Using M13 universal primer and T3 reverse primer, 220 bases from 5' end 272 bases from the 3' end has been sequenced. Four clones were sequenced which showed identical base sequences. Comparison with the published sequences of *P. falciparum* isolates from other countries revealed conformity as well as some distinctive features. Considerable portion at both the 5' and 3' ends has been found to be conserved with central polymorphic region as in other isolates. However, in Indian isolate central polymorphic region started many bases upstream in both the terminii. Internal primer has been designed to determine the rest of the sequence.

To PCR amplify the MSA 1 gene (1149 bp) primers corresponding to the 5' and 3' terminii were designed with an EcoRI site constructed in each primer and the amplified gene was cloned into the EcoRI site of PET-PL1-vector (Kumar, ICGEB). Considerable portion of MSA I gene has been sequenced with terminal primers and internal primers.

Effect of Heat-shock in *P. falciparum* Growth and Viability *in vitro*

Plasmodia undergo a heat-stress during malaria fever. Increase in temperature induces the synthesis of heat-shock proteins which are thought to perform functions that allow cells to surive at elevated temperatures. *P. falciparum* synthesizes four to five heat-shock related proteins, one of them is *Pf* HSP-70-1. This protein is expressed at all blood stages of the *Pf* including gametocytes. The antigenic part is composed of several tetrapeptide repeats of Gly-Gly-Met-Pro.

Five isolates of *P. falciparum* were synchronized at ring stage, cultured and then were exposed to 35°C, 37°C, 39°C and 41°C temperatures for 30 minutes. Cultures of each set were subjected to percoll density gradient, 10 per cent suspension of enriched parasites, having trophozoites and schizonts, were prepared in 0.15 M PBS, pH-7.2 and then sonicated and centrifuged. The soluble extract was tested for reactivity with antibody raised against recombinant HSP 70-1. Protein extract from one of the isolates showed high reactivity after heat exposure at 39°C (Fig. 15). Total RNA was isolated from this highly reactive isolate both before and after heat-shock at 39°C. It was then

Antigen preparation after heat treatment

hybridized with *Pf* HSP 70-1 probe. The signal was higher in case of parasite exposed to heat compared to control (Fig. 16). The results indicate that the *Pf* HSP 70-1 gene is heat inducible and enchancement may vary among different isolates.

Sera collections

A total of 500 serum/plasma samples from *P. vivax* infected patients and 102 samples from *P. falciparum* infected patients were collected and preserved.

Serum/plasma of rodent, avian and simian Plasmodia infected animals were also collected from the respective vertebrate hosts parasites and were cryopreserved (Fig 17). Different species of malaria parasites and sera were supplied to scientists of MRC and other institutes on request (Table 43).

In vitro* Cultivation of Exo-erythrocytic Stages of *P. vivax

Infections with *Plasmodium falciparum* and *P. vivax* are becoming more serious with the spread of insecticide and drug resistance. Hence, there is an urgent need to pursue new methods of control of this disease including vaccine development/immunoprophylaxis. Recent experi-

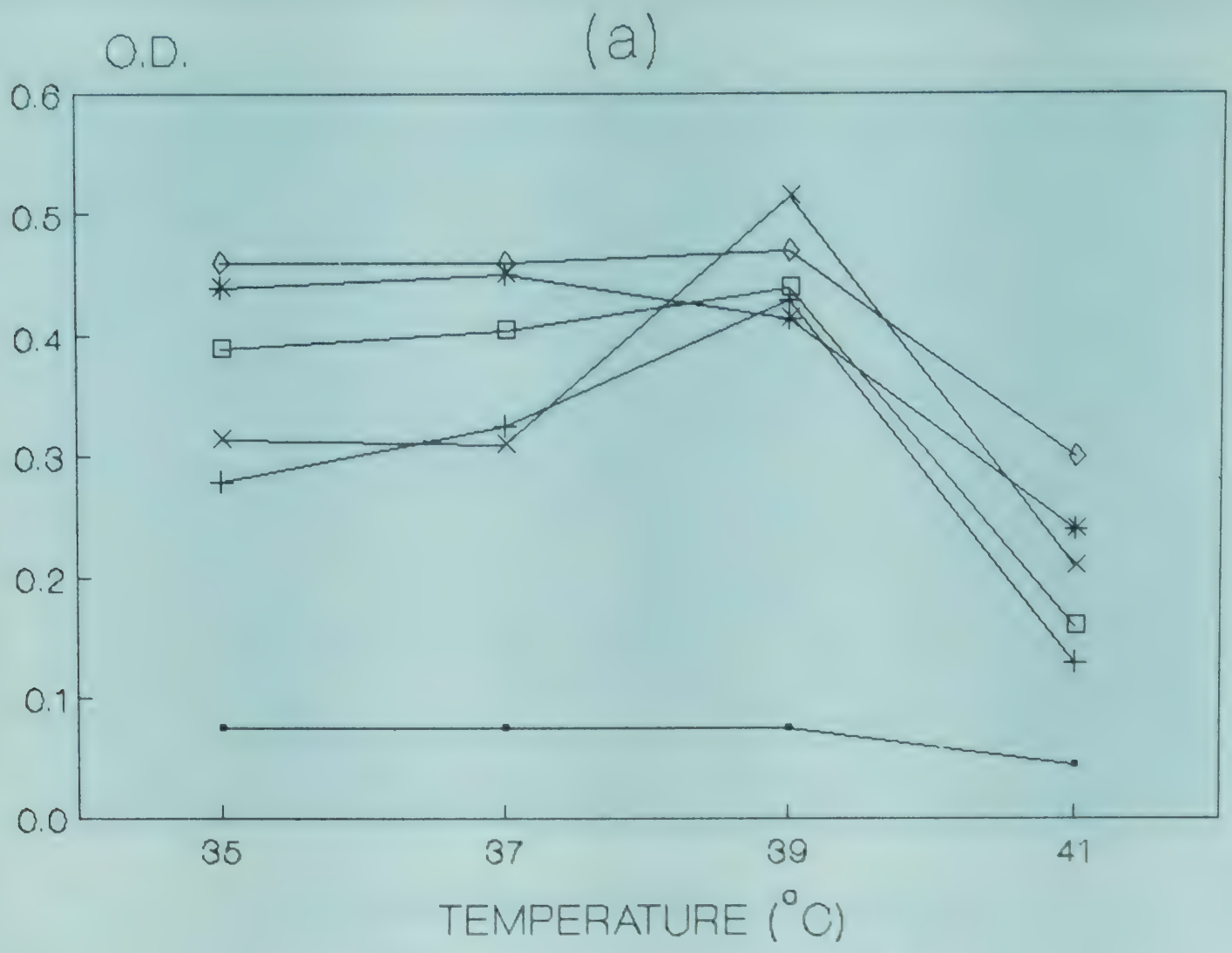
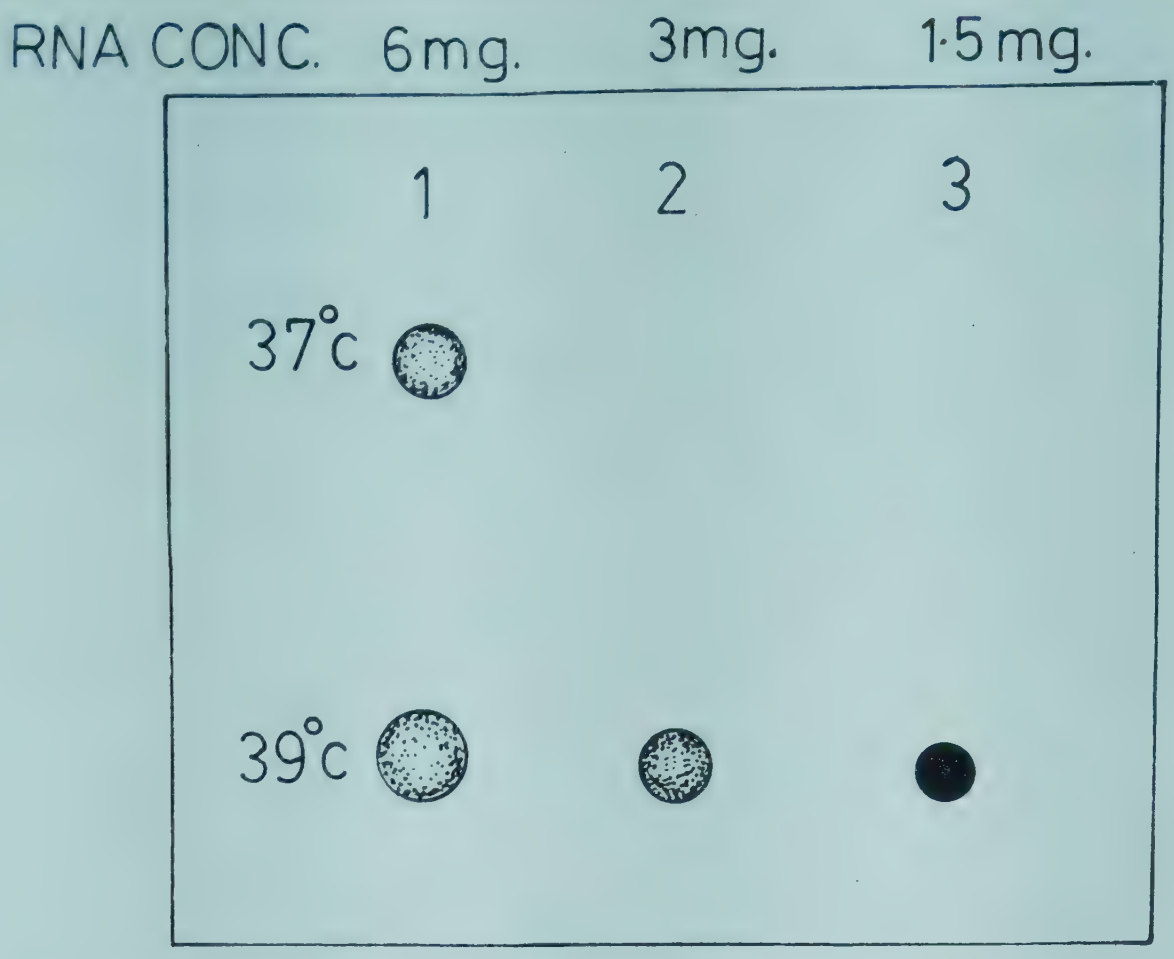


Fig. 15: Pf isolates show antigenicity after heat-shock tested against anti HSP 70-1 antibody raised in mice (a HC 44)



RNA Dot-blot analysis was done with various concentration of *Pf* RNA after 37°C temperature treatment. Hybridization was done with HSP DNA probe.

Fig. 16: Effect of heat-shock on expression of the *Pf* HSP 70-1 gene

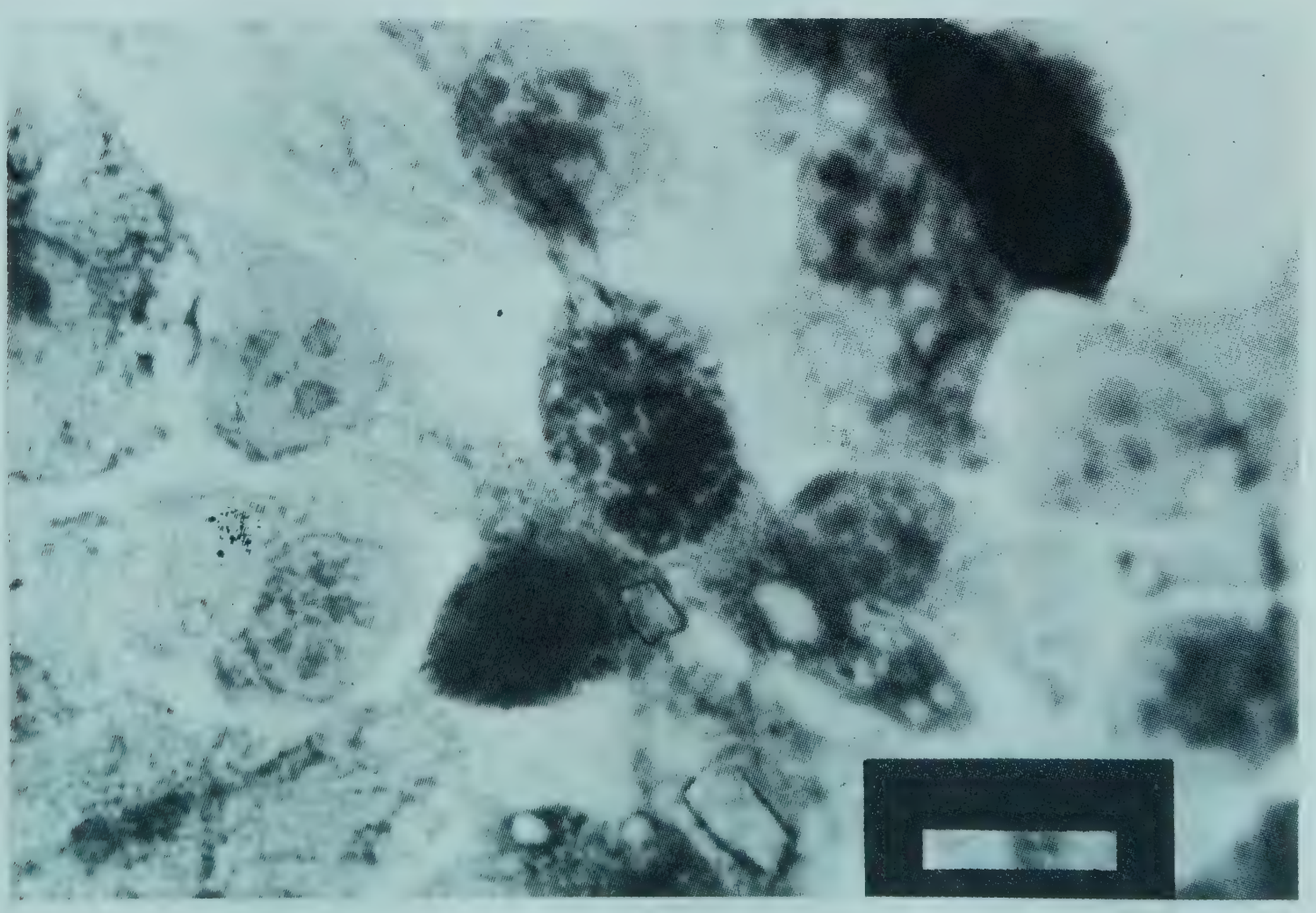


Fig. 17: Exo-erythrocytic stage of *P. vivax* (developing schizont) *in vitro*

Table 43. Major Research Institutes/Universities received biological materials from parasite bank during 1993

- All India Institute of Medical Sciences, New Delhi
- National Institute of Communicable Diseases, Delhi
- National Institute of Immunology, New Delhi
- Jawaharlal Nehru University, New Delhi
- International Centre for Genetic Engineering and Biotechnology, New Delhi
- Indian Institute of Sciences, Bangalore
- Post-graduate Institute of Medical Education and Research, Chandigarh
- Birla Institute of Technology and Sciences, Pilani
- Maharishi Dayanand University, Rohtak
- Institute of Microbial Technology, Chandigarh
- Central Drug Research Institute, Lucknow and
- Various divisions of Malaria Research Centre

ments have shown that the target of the effector mechanism induced by the sporozoites is not only the sporozoites but also the subsequent hepatic stages and the antigen expressed on the surface of the infected hepatocytes have an important role in inducing protection (Mazier, 1991).

To carry out this research, it is essential to establish an *in vitro* system for the cultivation of hepatic stage parasites since it is very difficult to get the material from malaria patients.

Techniques were standardized with hepatoma cell line (Hep G_2 A16) maintained in Williams' E. medium with 10% FCS. To infect these hepatoma cells, sporozoites were produced in *Anopheles stephensi* in the laboratory by artificial feeding on *P. vivax* infected blood. The sporozoites harvested aseptically from the mosquitoes were kept in an ice bath. The hepatoma cells, cultured in the lab-tek culture chambers and in 35 mm petri dishes were inoculated with desired number

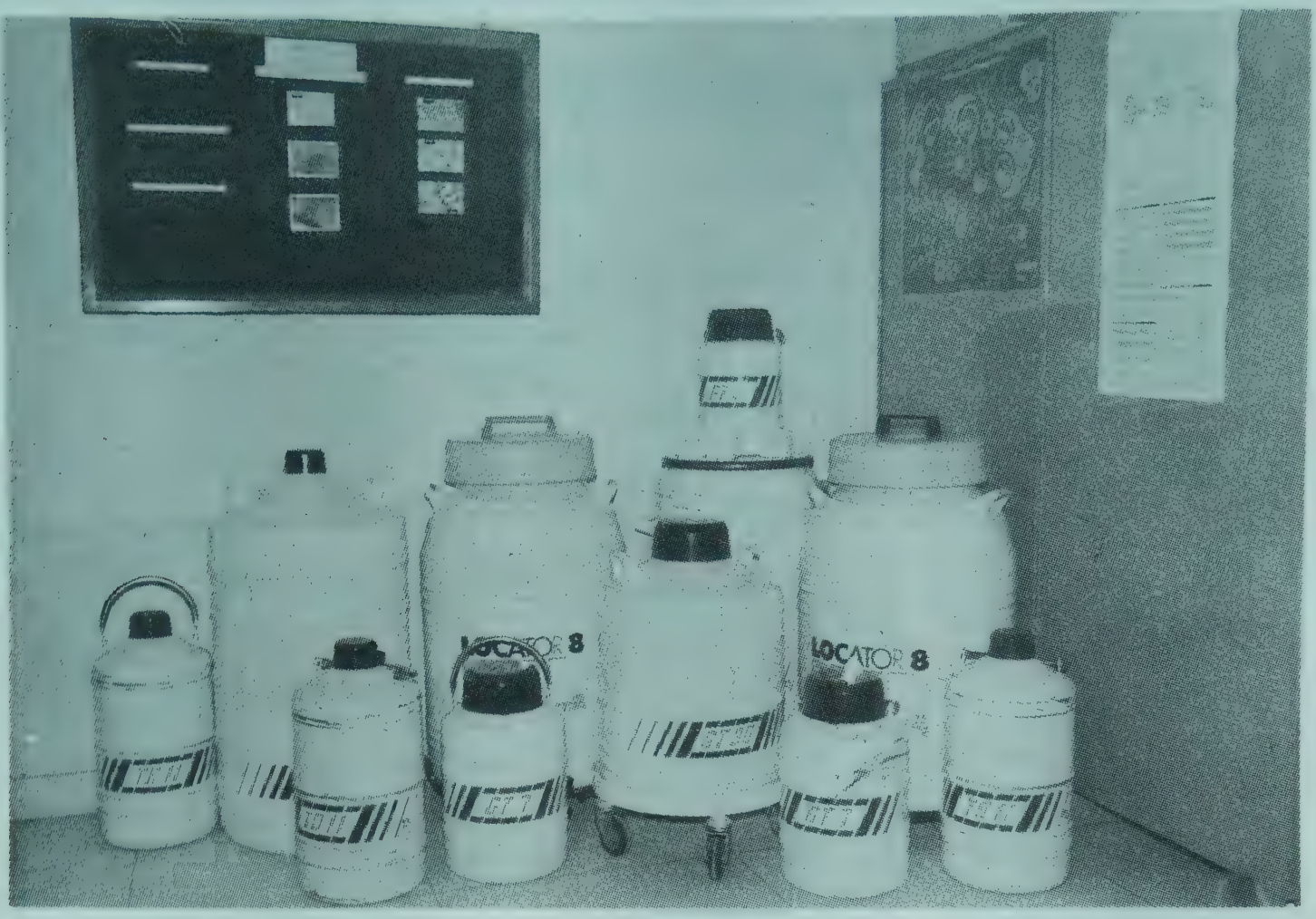


Fig. 18: Cryogenic containers used for the preservation of malaria parasites

of sporozoites. The culture was incubated for 4 hours at 37°C in a CO_2 incubator and the media was replaced with fresh complete Williams' E. medium. These sporozoite inoculated culutres were screened for 5-6 days for EE stage parasites. We were successful in getting exo-erythrocytic schizonts/developing schizonts *in vitro* (Fig. 18) for the first time in India. The aim is to inoculate the primary culture of normal human hepatic/parenchymal cells with mature salivary gland sporozoites for better yield of schizonts. Because of ethical reasons, normal human liver biopsy was not available for research purpose. Hence, the work was confined to hepatoma cell line. The infected hepatoma cells from petri dishes were harvested after 4-5 days and were kept frozen for purification of peptides/antigens for immunobiological studies.

Biochemical Characterization of Human Malaria Parasites

In continuation with the earlier work, more isolates were characterized for isoenzyme variations of GPI and GDH enzyme systems which are shown to exhibit good polymorphism. Table 44 gives the distribution of various alleles of GPI and GDH. Among the isolates about 85% comprised single clone and the rest 15% were multiclonal in nature. Average number of clones per isolate is about 1.1.

Table 44. Distribution of electrophoretic forms of GPI and GDH in *P. vivax* isolates

GPI			GDH		
Electrophoretic forms	Observed		Electrophoretic forms	Observed	
	Nos.	%		Nos.	%
I	92	66.7	I	90	78.9
II	34	24.6	IV	16	14.0
I+III	8	5.8	I+IV	5	4.4
I+IV	1	0.7	IV+V	2	1.8
IV	1	0.7	I+IV+V	1	0.9
IV+V	1	0.7			
VI	1	0.7			

There was no significant difference observed in the proportion of single and multiclonal isolates during low (April - July) and high (August - November) transmission periods (Table 45). Proportions of single and multiclonal isolates has not shown any statistical difference with respect to primary or repeated attacks of malaria.

Oocyst positivity of *An. stephensi* fed on these samples has shown that there was no significant difference observed in the oocyst range between isolates with single and multiclones. The range of oocyst varied from zero to 242 per gut.

Table 45. Clonal distribution during low and high transmission periods in Delhi

Period	Isolates with single clone (%)	Isolates with two or more clones (%)
Low transmission (Apr–Jul)	21 (46.7)	24 (53.3)
High transmission (Aug–Nov)	66 (61.1)	42 (38.9)

Table 46. Malate dehydrogenase polymorphism in *P. vivax* isolates

Electrophoretic forms	No. observed
I	15
II	5
III	1
IV	6
V	2
VII	8
I+II+VI	1
VI+VII	1
IV+VII	2
I+IV	7
Total samples	48

Another enzyme system MDH has also been studied in *P. vivax* isolates and it is found to be highly polymorphic. Table 46 presents the observed frequencies of various alleles of MDH and is very useful for characterization of isolates.

Cyclic Transmission of Rodent Malaria Parasites

In an attempt to explore the possibility of cyclic transmission of non-human malarial forms, studies were initiated with rodent malarial parasites. Maintainence of non-human malaria parasite in animals can serve as an excellent model to address intricate mechanisms of vector-host-parasite interactions.

Healthy Balb/c mice were selected for the study. They were maintained under standard laboratory conditions with free access to food and water. Mice were divided into two groups. Each group was inoculated (intraperitoneally) with respective parasites such as *P. yoelii yoelii* and *P. yoelii nigeriensis* obtained from INSERM, Paris and LSHTM, London respectively. The blood smear of each mice was prepared and stained with JSB solution. Parasites were observed in the peripheral blood from 3-4 days onward (Figs. 19 and 20). Gametocyte positive mice were placed in a restrained cage and presented to

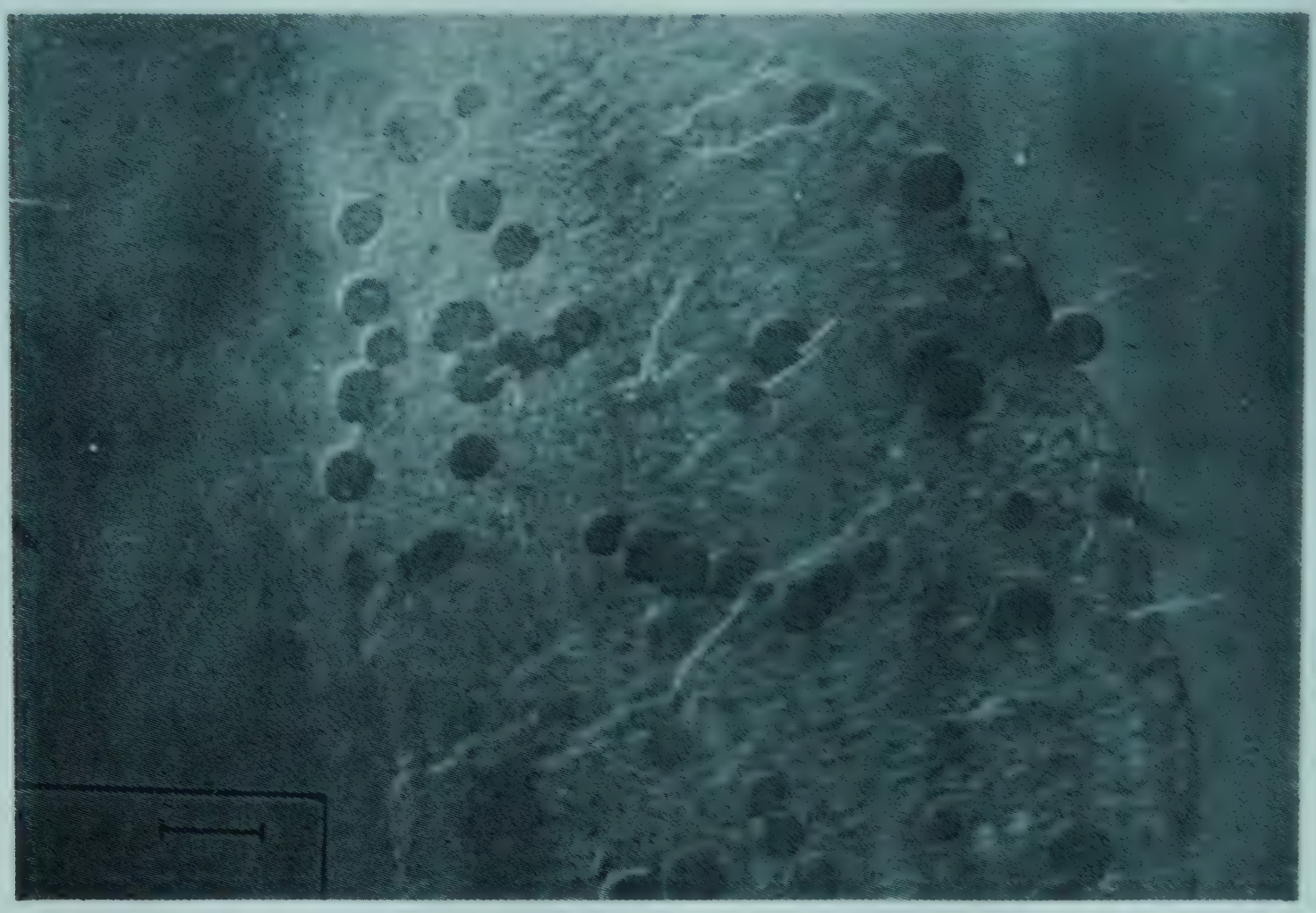


Fig. 19: Oocysts of *Plasmodium yoelii nigeriensis* on the gut of *An. stephensi* on the 11th day. 153 x

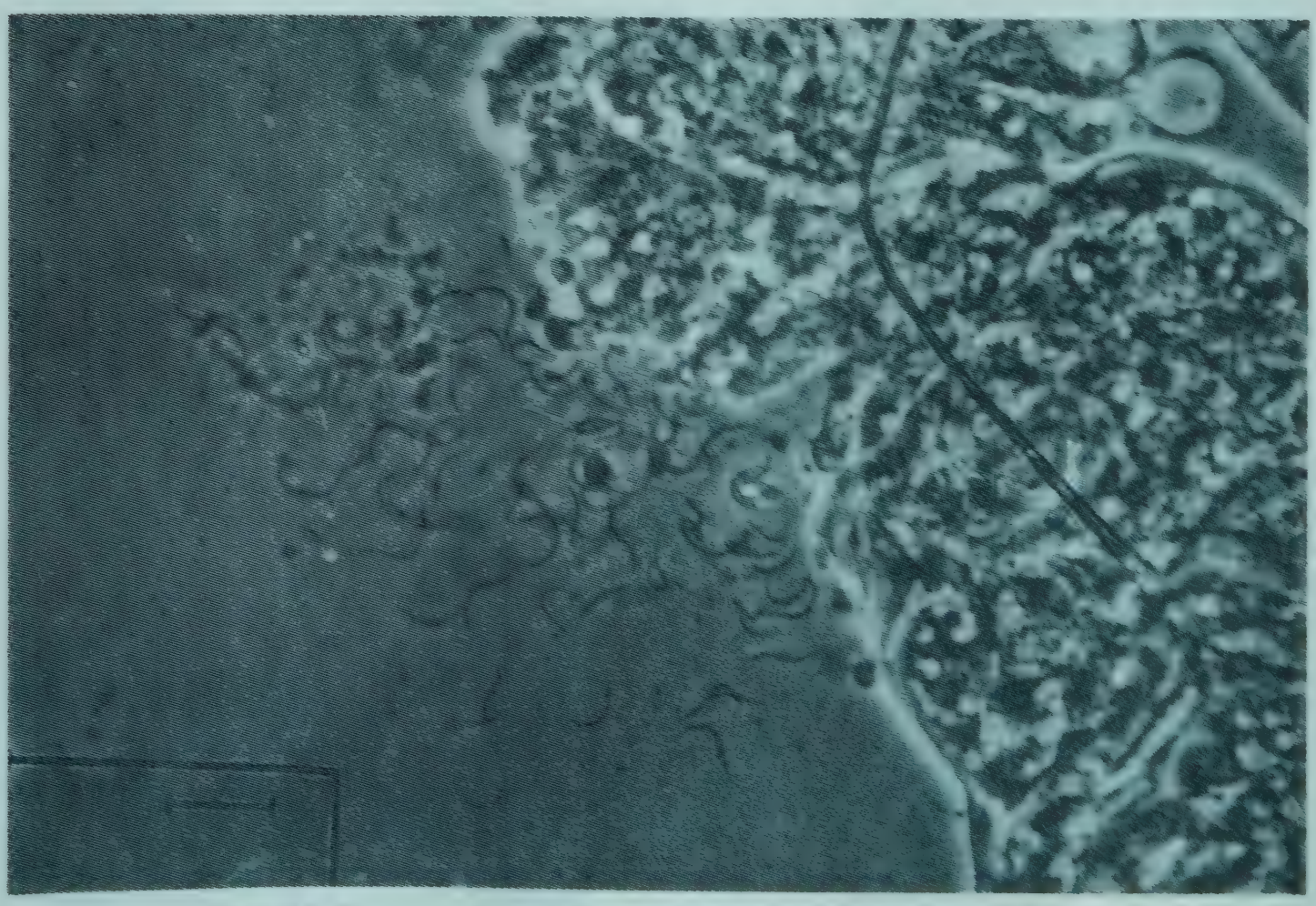


Fig. 20: Sporozoite of *P. yoelii nigeriensis* in the salivary gland of *An. stephensi* on the 11th day. 1530 x

previously starved *An. stephensi* for 1 h. During the feeding process, some of the full-fed mosquitoes were aspirated out and a smear was prepared to check for the exflagellation. After the feeding period, the full-fed mosquitoes were maintained at 24°C±1. The infectivity rate was monitored by dissecting out the gut and salivary gland for the presence of oocyst and sporozoites respectively. The mosquitoes from sporozoite positive batch were fed to normal mice. Post-prandial dissection was done to ascertain the presence of sporozoites in the salivary gland. The mice were maintained under standard laboratory conditions. They were positive for the parasite from 8-10 days onward. Studies would be undertaken with other rodent malaria parasites.

Oxidant Defense: Superoxide Dismutase (SOD) and Catalase in *Plasmodium vivax*

Invasion of the human erythrocytes by malaria parasites is accompained by a variety of biological responses in the host. The developing malarial parasite have considerable potential to bring about metabolic changes in the host cell, and become more vulnerable to damage due to toxic metabolites derived from both the host and the parasite. One of the notable features of malaria infection is the activation of the cellular immune system and production of reactive oxygen species (ROS). During the respiratory burst, phagocytic cells release superoxide ions (O^-), hydrogen peroxide ($H_2 O_2$) and hydroxyl radicals (OH^-). The ROS are toxic to parasites and have the potential to bring about host tissue damage. Thus ROS are implicated both in protection and pathogenesis of malaria. High levels of SOD and catalase have been reported in cultured erythrocytes infected with *P. falciparum*. The murine malarial *P. berghei* and human malaria parasite, *P. falciparum* have been shown not to produce their own SOD, an enzyme which is important in the protection against activated oxygen species. This lack of endogenous SOD is counter balanced by the adoption of high levels of host SOD within the parasite. However, till date, there is no information on these protective enzymes, SOD and catalase in *P. vivax*.

Parasites were isolated from *P. vivax* infected patient's blood by lysis with 5 mM phosphate buffer (pH 8). The parasites were isolated with relatively little contamination from the host red cell cytoplasmic materials. Most of the glutamate dehydrogenase activity, which is considered to be a marker of parasite, was found to be concentrated in the isolated parasites. However, the specific activity of catalase was found to be about 17% of that present in the host cell cy-

toplasm (Table 47). Parasites sub-cellular fraction were obtained by differential centrifugation. The distribution of enzyme activities in the various fractions are shown in Table 48. These data are expressed as relative specific activities also in Table 49 for hemoglobinase, SOD and GDH.

Table 47. Percentage of total recovered glutamate dehydrogenase and catalase in isolated *P. vivax* parasites

Enzyme	% recovered in parasites*
Protein	5.62 ± 1.60
Catalase	0.97 ± 0.32
Glutamate dehydrogenase	78.30 ± 10.82

Values are mean ± SD; * $\frac{\text{parasite}}{\text{parasite + red cell supernatant}}$ x 100

Table 48. Percentages of enzyme activities in 10 K, 100 K and soluble *P. vivax*

Fraction	Total protein	Hb' ase	GDH	SOD	Catalase
10 K	25.3 ± 3.90	47.9 ± 3.2	15.4 ± 3.8	55.8 ± 12.5	0.48 ± 0.16
100 K	18.4 ± 2.10	24.2 ± 4.3	2.3 ± 1.6	27.5 ± 8.7	0.45 ± 0.09
Cytosol	56.3 ± 4.20	27.9 ± 9.6	82.3 ± 18.9	16.7 ± 9.6	ND

ND = Not detected; values are mean ± SD of five experiments in triplicate

Table 49. Relative specific activities of Hb'ase, glutamate dehydrogenase and superoxide dismutase in sub-cellular fractions

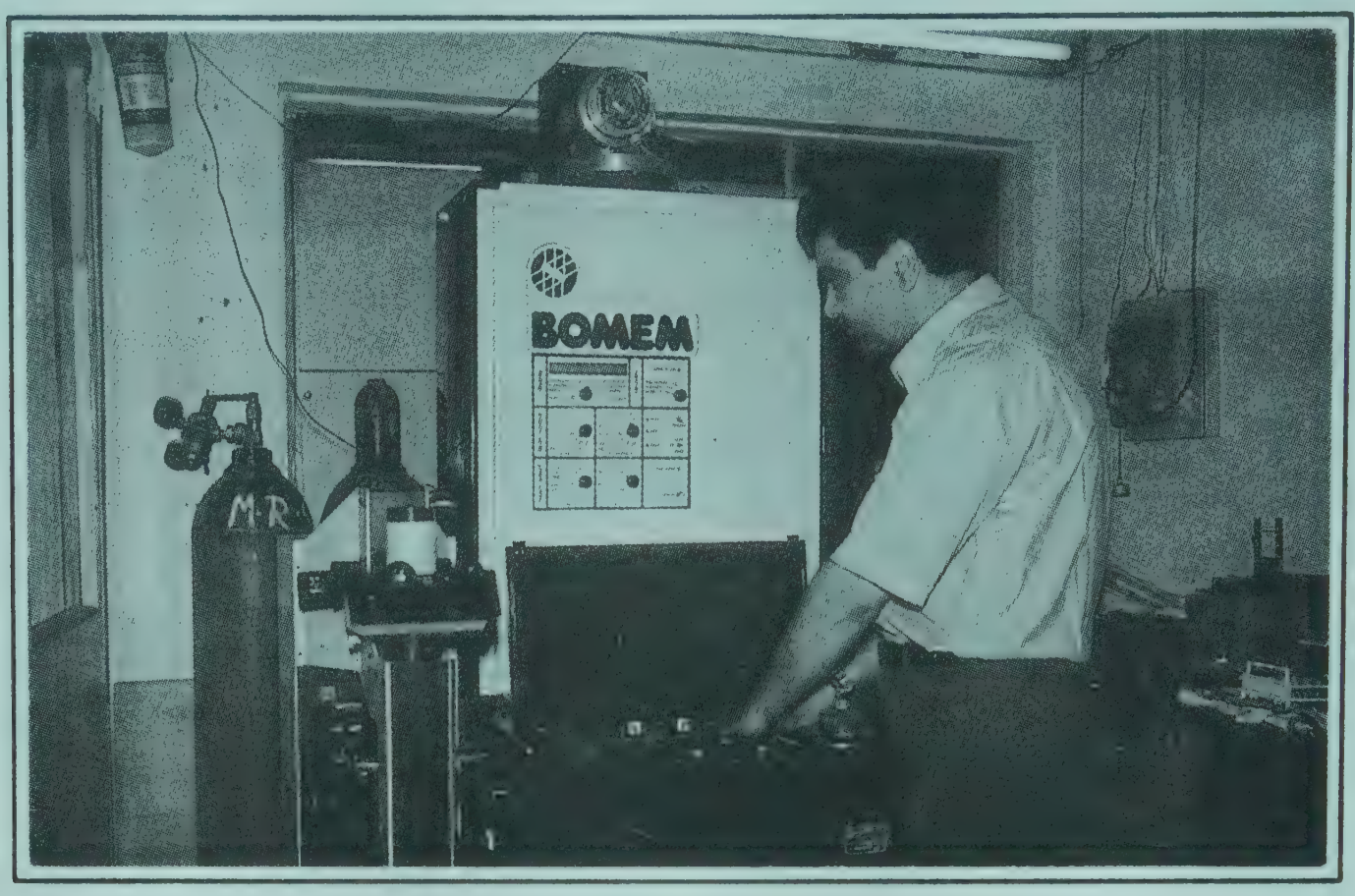
Fraction	(Values are mean ± SD of 5 replications) units/ mg protein		
	Hb'ase	GDH	SOD
10K	1.89 ± 0.08	0.607 ± 0.24	2.20 ± 0.33
100 K	1.31 ± 0.48	0.120 ± 0.08	1.49 ± 0.92

Most of the GDH activity was found to be concentrated in the cytosolic fraction of the parasite as observed by per cent activity and also by relative specific activity whereas, the SOD activity was found to be concentrated mostly in the 10 K and 100 K pellets. The 10 K and 100 K pellets were identified as the lysosomal fractions by measuring the activity of acid hemoglobinase which is considered to be the marker of lysosomal fractions. Total activity 72% of acid hemoglobinase was found to be in 10 K and 100 K fractions. The relative specific activity (1.89 and 1.31 respectively) was about 3 times than the specific activity of this enzyme in cytosol. SOD activity followed almost the same pattern as hemoglobinase. The total activity of SOD in the two pellets was calculated to be about 90% of the activity found in the cytosolic fraction (Tables 48-49). Sub-cellular localization of the catalase activity was also followed in various parasite fractions. It has been observed that most of the activity of catalase was sedimented with cellular debris and that is pelleted at 2250 g. In the cytosolic fraction of the parasite however, we were not able to detect any catalase activity (Table 48).

In this report two oxidant defense enzymes have been studied in *P. vivax*, SOD and catalase. Till date, there is no evidence that SOD or catalase is produced by malarial parasites. The enzyme might be pinocytosed by the parasite along with hemoglobin and other erythrocytic cytosolic proteins. Once the host SOD enters the parasite cytosol it gets concentrated in the 10 K and 100 K fractions which is reported to be lysosomes. Inside the lysosomes it may resist the lysosomal enzymes and remain as defensive mechanism against free radical attack. The parasite is normally shielded in the erythrocytes from external oxidative agents. Erythrocytes are very rich in the oxidant defense enzymes. Most of the antimalarial drugs, which are believed to act via the prodution of free radicals are believed to exert pressure only because they can bypass these external oxidant defense mechanisms. Why the parasite might live so dangerously, is not known at present. However, there are some evidences that parasite might have an enzyme which is not cyanide sensitive and thus may be different from human SOD. This aspect to define various enzyme forms of SOD, fractions and also the potential in chemotherapeutic use, if any, in malarial infections is currently under investigation.

FT-IR Spectra of Human Malarial Parasites

Fourier-transform infrared spectroscopy (FT-IR) was applied to the study of human malarial parasite lysates. Pairs of samples of *P.*



FT-IR spectroscopy

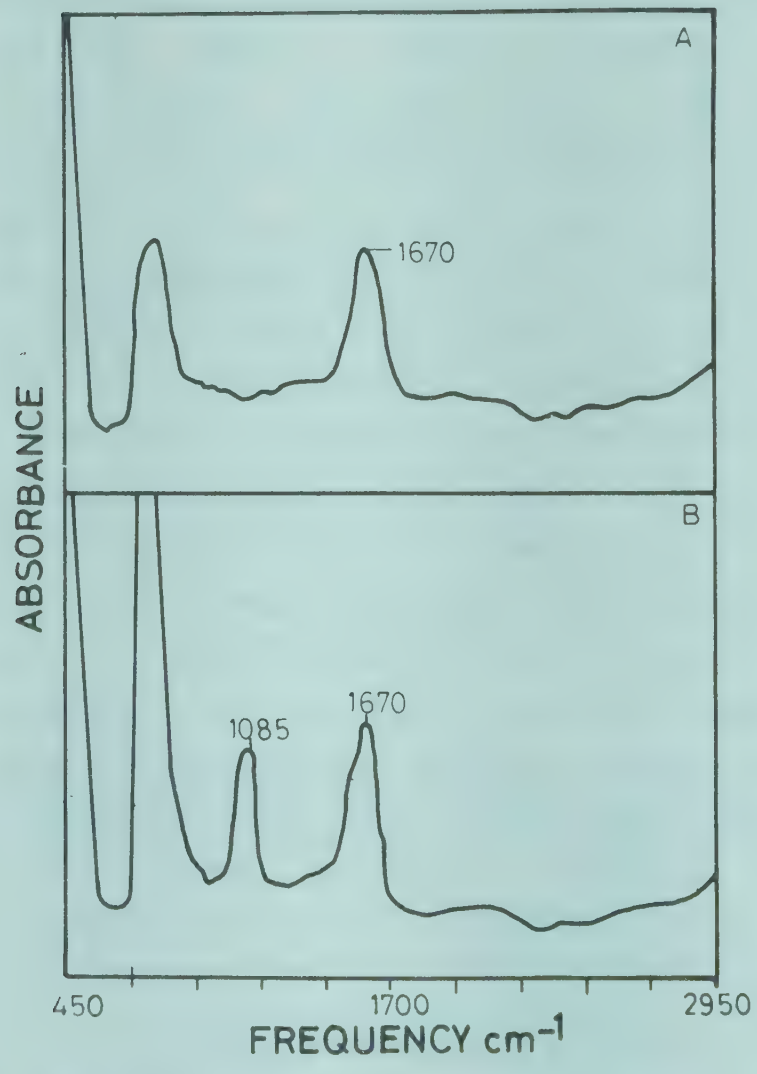


Fig. 21: FT-IR spectra in the frequency region of 450-2950 cm^{-1} of a normal phosphate buffer (A) and *P. vivax* lysate (B)

falciparum and *P. vivax* were obtained from patients who had attended the malaria clinic. All samples displayed specific spectra compared to the corresponding normal samples/phosphate buffer. The spectrum contains the amide I, II and III bonds characteristic of the protein secondary structure near 1650, 1550 and 1250^{-1} cm respectively. These specificities involved the phosphate and C-O stretching bonds, and CH stretch region. The parasite proteins responsible for these spectral specificities are unknown. Efforts are being made to establish the diagnostic potential of these observed spectral changes for *P. falciparum* and *P. vivax* at molecular levels under physiologically relevant conditions (Figs. 21, 22).

Non-human Plasmodia

In addition to the human malaria parasites, there are large number of different species of plasmodia infecting various animals and birds. Since these animals are easy to handle, they can be used for various experiments especially in antimalarial drug testing in malaria infected animals and for immuno-biological studies. The parasites can be maintained in these animals by infected blood passages or through the vector mosquitoes.

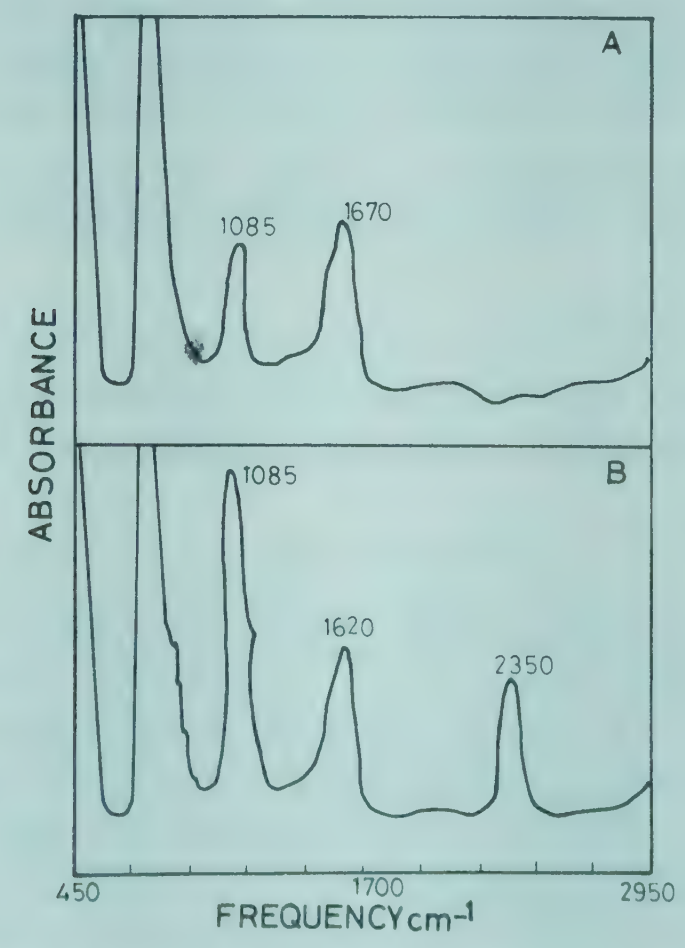


Fig. 22: FT-IR spectra in the frequency region of 450-2950 cm^{-1} of *P. falciparum* (B) and *P. vivax* lysates (A)

Studies on Reversal of Chloroquine Resistance

There is ample evidence documenting the increasing incidence of malaria worldwide, largely due to the spread of *P. falciparum*. The deteriorating efficacy of existing antimalarial drugs, is because of increasing number of drug resistant parasite strains which make routine prophylaxis and treatment of the disease a therapeutic challange. In view of these problems efforts are needed that will lead to the discovery and development of new drugs in several formulations effective against all developmental stages of four human parasites and have few unacceptable side effects. It is not unreasonable to believe that novel compounds with potential antimalarial activity could be found through an organized screening effort. So the research focus in our studies was to identify plants/compounds and screen them for antimalarial activity systematically in pre-clinical and clinical experiments.

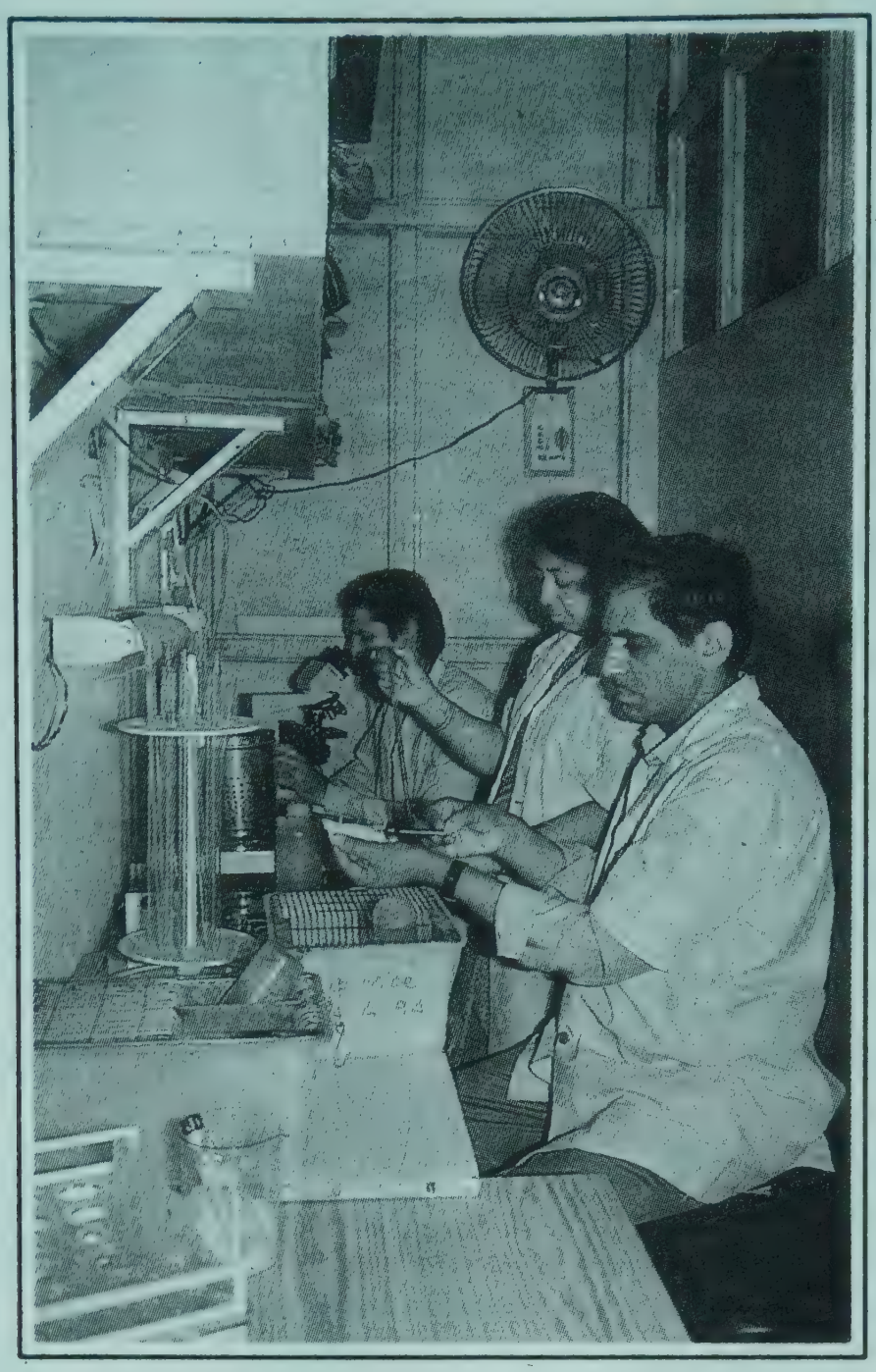
Screening of drugs/chemicals, indigenous compounds for antimalarial activity

Neem oil: Neem (*Azadirachta indica*) is an indigenous tree growing in India. Many medicinal properties have been attributed to this tree since ancient times. Hot water extract of neem was reported to cure malaria. The results of various studies on antimalarial activity in animal models are contradictory. Results vary from encouraging activity of leaf extracts to mild or no activity of sodium nimbinidate in *P. berghei* malaria.

Experiments were thus undertaken to explore the antimalarial activity of neem oil fractions *in vitro* on *P. falciparum* and *in vivo* on *P. berghei* in mice.

***In vitro* studies:** Studies were carried out with chloroquine sensitive (FSJ-A6) adapted strain of *P. falciparum*. After adjusting the parasitaemia at 1-1.5%, the parasites were exposed to various concentrations of neem oil fractions 1 and 2 for 24 h in parallel with control cultures (neem oil fractions were prepared with the help of HPLC at Hardwar field station).

Parasite growth was evaluated in JSB stained blood smears by counting number of parasites per 200 asexual parasites. Per cent inhibition of schizont maturation was then calculated (Table 50).



Screening of drugs for antimalarial activity in animal model

The results indicate that fraction 1 does not have antimalarial activity even at high concentration of 100 µg/10 µl of blood. Fraction 2 has IC_{50} values of approximately 10 µg/10 µl of blood in this model.

***In vivo* studies:** Mice (20-25 g) were inoculated with chloroquine sensitive strain of *P. berghei* on day 0. They were allowed food and water *ad libitum*. Treatment was then given with neem oil fractions at various doses subcutaneously for 4 days consecutively. Parasitaemia was determined on day 4 and compared with control group (Table 51). Doses lower than 100 mg/kg were not effective. Doses above 200 mg/kg produced acute toxicity. Wider range of dose levels could not be screened because of limited quantity of compound.

The results indicate lack of activity against asexual blood forms of *P. berghei* in this model.

Table 50. Antimalarial activity of neem oil fractions *in vitro*

Pf isolate FSJ–A6 (chloroquine sensitive)		
Conc. of fraction (µg/well)	% Schizont maturation inhibition	
	Fraction 1	Fraction 2
100	17.65	100
50	0	96
25	–	74.5
10	–	52.94
5	–	51
5	–	37.25
2.5	–	21.52

Table 51. Effect of neem oil fractions on chloroquine sensitive *P. berghei* in mice *in vivo*

Drug (S.C. x 4 days)	Dose	Parasitaemia (day 5)
Control	–	5.06 ± 0.25
Chloroquine	3 mg/kg (base)	0
Neem oil fractions		
1 (in methanol)	100 mg/kg	4.6 ± 0.38
	200 mg/kg	4.94 ± 0.88
2 (in ethylacetate)	100 mg/kg	3.88 ± 0.52

Antimalarial activity of extracts of an indigenous plant from M.P.: Five extracts of a wild herb (local name *Bhuineem*) from District Bastar, Madhya Pradesh were screened for antimalarial activity. The plant is used for treating malarial fever by local people. *In vitro* studies were carried out with FSJ-A6 strain of *P. falciparum* after dissolving five fractions of Bhuineem in methanol or chloroform. Final dilution was done with RPMI 1640 medium. Per cent schizont maturation inhibition is shown in Table 52.

Preliminary results indicate that fractions 1-4 have IC_{50} in the range of 10-25 µg/10 µl blood. Thus there is evidence of antimalarial activity.

In vivo studies with chloroquine sensitive strain of *P. berghei* in mice are in progress to confirm the antimalarial activity in animal model.

Antimalarial activity of flouroquinolones: Several antibiotics have antimalarial action. Flouroquinolones have been reported to be active against chloroquine sensitive and chloroquine resistant strains of *P. falciparum in vitro*. 100% cure rate was reported with norfloxacin in 9 patients from Madhya Pradesh. However, negative results were obtained by a number of workers in different parts of the world. We report approximately 30% efficacy with 400 mg BD dose of norfloxacin for 3 days in *P. vivax* and *P. falciparum* infections. Efficacy of double dose was 100%. As ciprofloxacin is more potent than norfloxacin, we studied antimalarial activity of ciprofloxacin alone and in combination with chloroquine at different doses in chloroquine resistant strains of *P. berghei* in mice.

Balb(c) mice were administered 1 x 10^6, PRBC (chloroquine resistant strain) and different doses of drugs were given subcutaneously for 4 consecutive days. Parasitaemia was determined on Day 5 and compared with control group (Table 53).

Table 52. Antimalarial activity of fractions G1–G5 *in vitro* in FSJ–A6 (chloroquine sensitive) isolate of *P. falciparum*

Conc. (µg/well)	% schizont maturation inhibition				
	G1	G2	G3	G4	G5
100	100	85.7	88.2	88.5	25.5
50	84.3	80.0	80.3	88.5	1.96
25	58.8	71.4	63.7	51.4	1.96
10	47.06	22.8	21.5	25.7	0
5	37.2	14.2	21.5	14.2	0
2.5	21.5	11.4	11.7	0	0
1.25	13.7	2.8	5.8	0	0

Table 53. Effect of ciprofloxacin (cipro) on chloroquine (CQ) resistant *P. berghei* in mice that recieved ciprofloxacin alone or in combination with chloroquine

Drug (S.C. x 4 days)	Per cent parasitaemia		
	Day 6	Day 8	Day 10
Control	1.61 ± 0.44	2.5 ± 0.3	16.72 ± 1.54
CQ 5 mg/kg	1.56 ± 0.13	2.9 ± 0.2	12.4 ± 0.72
CQ 5 mg/kg + cipro 10 mg/kg	1.38 ± 0.24	2.4 ± 0.13	12.4 ± 0.62
CQ 5 mg/kg + cipro 30 mg/kg	1.95 ± 0.41	2.87 ± 0.34	21.75 ± 5.85
Cipro 100 mg/kg + CQ 5 mg/kg	0.53 ± 0.15	1.61 ± 0.36	14.8 ± 1.97
Cipro 30 mg/kg	1.7 ± 0.7	4.4 ± 0.49	18.8 ± 2.32

The results indicate that ciprofloxacin is not an effective blood schizonticidal for chloroquine resistant strains of *P. berghei* either alone or in combination with chloroquine.

Phase II clinical trial with compound 80/53: Antimalarial drugs such as Primaquine are known to cause haemolytic episodes in subjects having G-6-PD deficiency. A compound 80/53 {N'-(3-Acetyl-4-5-dihydro-2-Furanyl)-N-(6-methoxy-8-quinolinyl) 1,4-peritanediamine} has been developed by CDRI, Lucknow as an anti-relapse antimalarial. Data shows that efficacy is comparable to Primaquine and toxicity is less. Phase I clinical trial has confirmed the safety of the compound.

To evaluate the anti-relapse efficacy of compound 80/53 in patients of *P. vivax* malaria, phase II trial is being conducted at MRC in collaboration with CDRI and NMEP. Prospective, Randomised, double blind, non-crossover, comparative trial as per declaration of Helsinki is in progress.

After obtaining informed written consent and carrying out complete physical examination patients were enrolled in the study according to inclusion criteria. Pre-treatment investigations viz: Hb, TLC, DLC, Urine (microscopic, albumin, sugar) and peripheral smear for malaria were carried out. Chloroquine was then given (1500 mg over

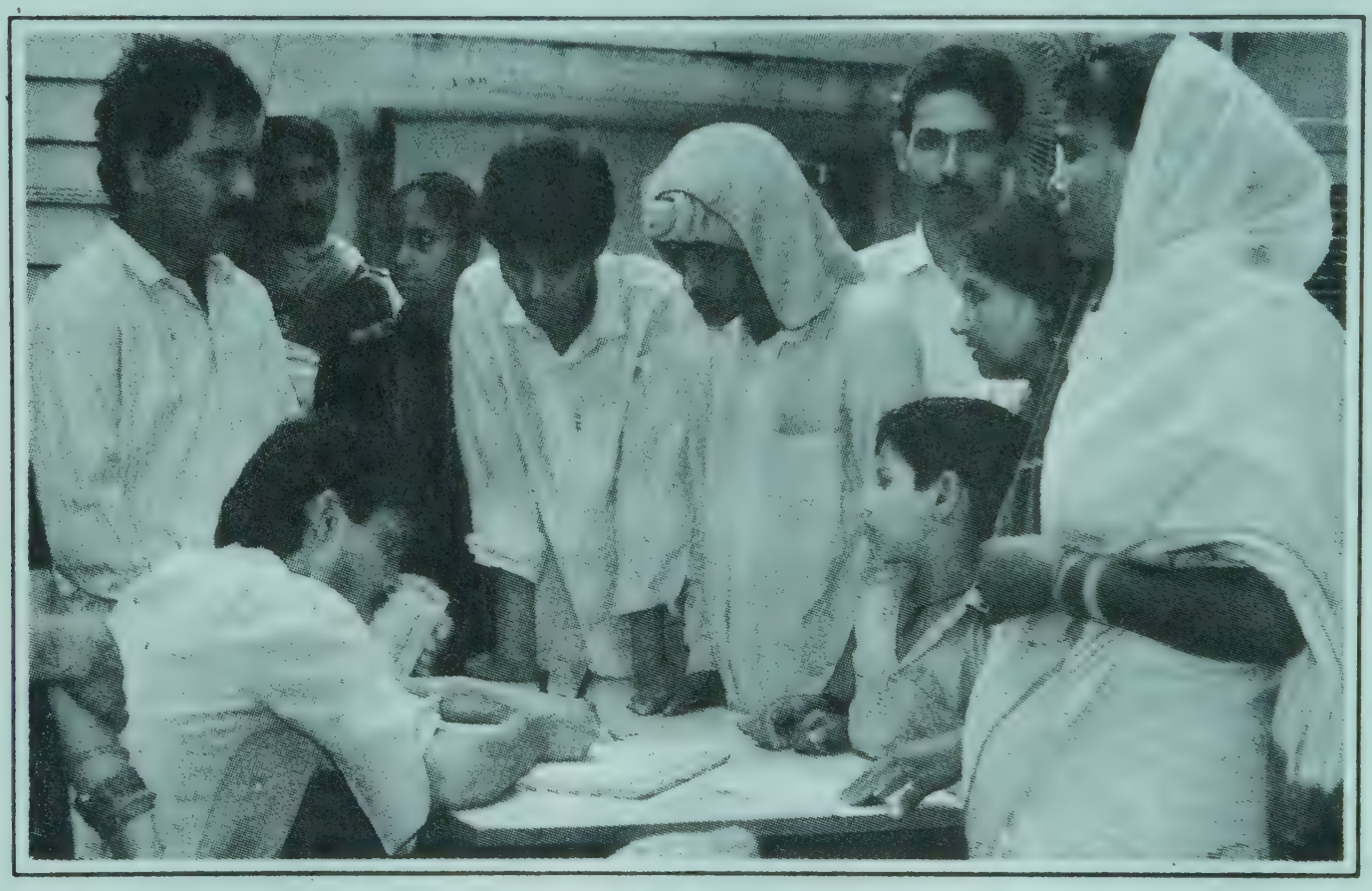
3 days) along with symptomatic treatment. Then the patients were assigned one of the three treatments viz. placebo, primaquine or 80/53 in double blind manner. Post-treatment investigations were done after 5 days. Follow-up is being carried out fortnightly.

Till date at 22-Sham Nath Marg clinic, a total of 99 cases have been enrolled. Out of these 15 were excluded after investigations. 3 cases dropped out. 7 patients reported back with 2nd episode of *P. vivax* and follow-up is continuing in rest of the 74 patients.

Similar trials are also in progress at Malaria Clinic, Nanak Enclave. Here 423 patients have completed the therapy using chloroquine (WHO regimen) along with drug-capsule. 45 patients returned with fever and malaria after the completion of five days treatment. 11 are untraceble and 7 left drug trial on medical/technical grounds. The work of follow-up of the trial patients is still in progress.

Malaria Clinic, Delhi

During 1993 a total of 5523 patients visited the malaria clinic at Malaria Research Centre, Nanak Enclave. Of these, 2243 cases were malaria positive including 2186 cases of *P. vivax*, 55 cases of *P. falciparum* and 2 cases of mixed infection. The slide positivity rate (SPR) and slide falciparum rate (SfR) for the whole year were 40.61 and 1.03 respectively (Table 54).

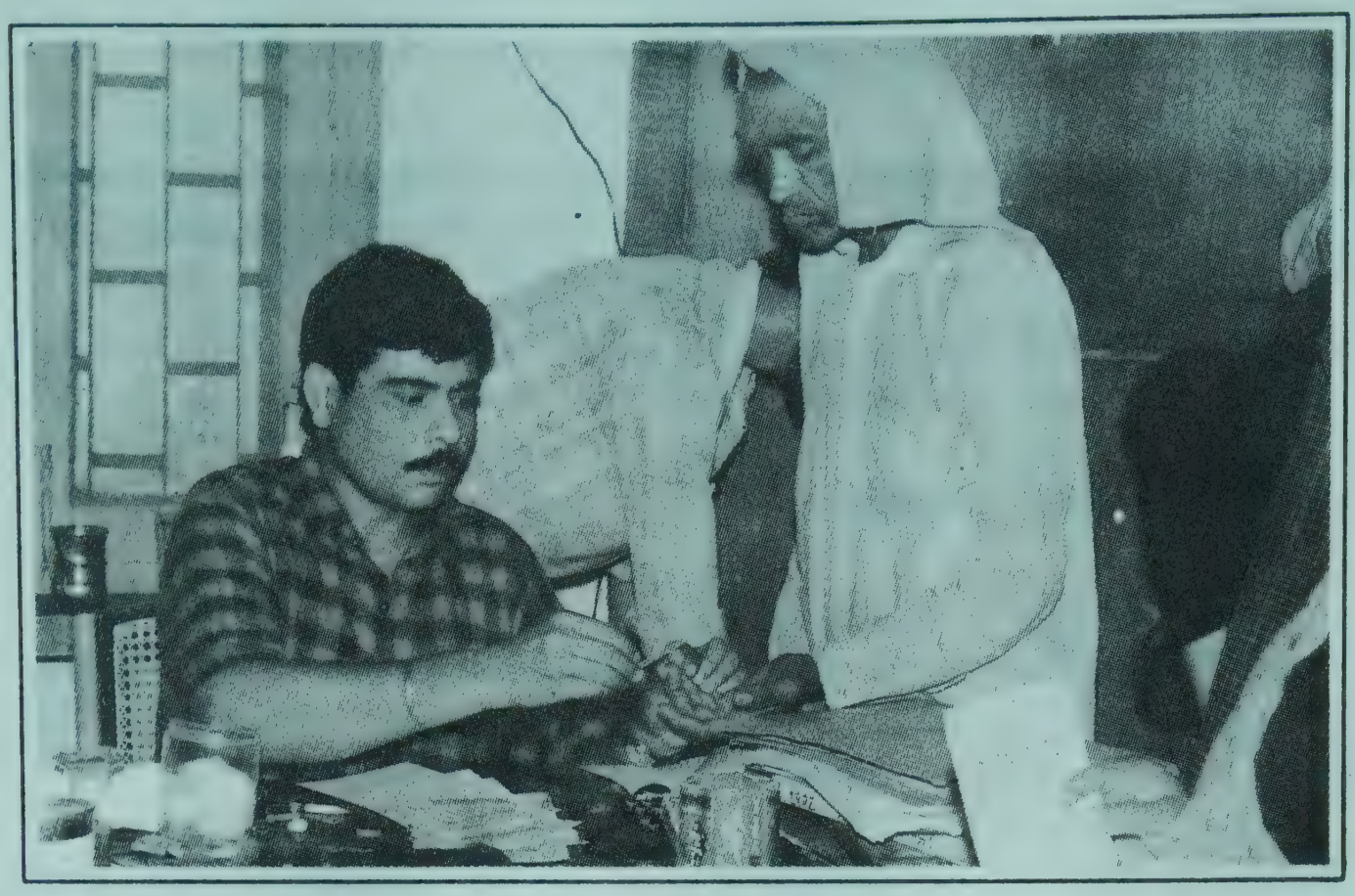


Patients giving their particulars and fever history

Other Clinical Studies

During the screening of patients for the drug-trial, an attempt was made to go into the details of clinical presentation and G-6-PD deficiency status of these patients. An observation based on the compilation of results of over few hundred patients revealed that headache (frontal area) was visibly a prominent symptom among these patients.

Also a detailed account of the clinical symptomatology of patients attending the malaria clinic revealed that the seasonal infections did play a role in it. A mimicry was found in the clinical symptomatology



Blood slide is being made

Table 54. Data from malaria clinic (1993)

Month	BSE	Total +ve	*Pv*	*Pf*	Mix	SPR	SfR
Jan	56	17	13	4	–	30.35	7.14
Feb	113	24	22	2	–	21.23	1.76
Mar	129	36	33	3	–	27.91	2.32
Apr	189	52	52	–	–	27.51	–
May	241	87	87	–	–	36.10	–
Jun	298	139	138	1	–	46.64	0.33
Jul	672	160	160	–	–	23.80	–
Aug	839	349	347	2	–	41.59	0.23
Sep	1199	608	599	9	–	50.71	0.75
Oct	1067	457	440	16	1	42.83	1.59
Nov	536	255	243	11	1	47.57	2.23
Dec	184	59	52	7	–	32.06	3.80
Total	5523	2243	2186	55	2	40.61	1.03

between vivax malaria patients and patients with upper respiratory tract infections/other mild viral/seasonal infections.

An observation with regard to the fluctuations in haemoglobin-status among the malaria patients revealed a rise in haemoglobin-level in a good proportion of cases. The reasons for this are yet not known to us. Therefore, the findings were repeated among fever patients without malaria in order to affirm or deny our observation.

Monitoring of Haptoglobins in Malaria Patients

In continuation to our earlier work on the role of genetic markers in relation to malaria endemicity, samples from a migrant labour camp population in South Delhi were analyzed for haptoglobin (Hp) polymorphism using polyacrylamide gel electrophoresis.

Table 55 presents the distribution of Hp polymorphs. Results have shown that among the typable samples, 80% were of Hp 2-2 type and 20% were of 2-1 type. A large proportion of samples could not be typed due to the absence of readable pattern on polyacrylamide gel. However, the proportion of non-typable samples was significantly higher in *P. falciparum* positive subjects than the negative control (Chi square value 13.24, df=2, $P < 0.01$).

Haptoglobin analysis has also been done from patients visiting malaria clinic, NMEP, Delhi on pre- and post-chloroquine therapy to know after what period of time, typable haptoglobins are recorded in patients with non-typable Hp 0 (ahaptoglobinemia). Table 56 presents the data on haptoglobin analysis on HpO patients after chloroquine therapy. It is seen from the Table 55 that by 8-9th day most of the

Table 55. Haptoglobin polymorphism in a representative sample of a labour camp population

Group	No. tested	Haptoglobin phenotypes		
		2–2	2–1	NT
Malaria (–ve)	25	13	4	8
Pf +ve	24	3	1	20
Pv +ve	4	4	0	0

NT – non typable

Table 56. Data on ahaptoglobinemic subjects after chloroquine treatment

Days after infection with chloroquine therapy	No. of samples tested	No. of samples showing typable Hp phenotypes
6	1	1 (2–2)
8	8	6 (2–1, 2–2)
9	3	2 (2–2)
11	2	0
12	2	1 (2–2)
13	2	0
16	1	1 (2–2)

samples (75%) show typable haptoglobin levels. However, in some of the samples typable Hp forms could not be observed till day 13.

Malariogenic Stratification of Delhi

The study of malariogenic stratification in Delhi was initiateed in 1989. Quantitative data on malaria incidence, entomological data and ecological variables pertaining to physiogeography, hydrology, demography, human settlement density and water pollution parameters were collected from eight representative localities for two years.

Stratification models were developed based on these variables. Only statistically significant ecological variables which explained more than 80% variation due to regression in malaria indicators were selected using stepwise regression method. Based on stratification approach, each malaria zone (stratum) of Delhi was identified and classified into three types to understand the distribution of malaria and the potential of malaria transmission.

High epidemic risk type: This includes sites of labour aggregations, scattered colonies, developing areas and Jhuggi Jhopri (JJ) clusters with estimated annual parasite index (API) above 10. These areas are expected to have localised outbreak of malaria epidemics with a potential to spill over to the neighbouring areas. Therefore, in these situations suitable adulticide spraying, running malaria clinics and prompt treatment of malaria cases to reduce/eliminate the gametocyte load in the community would be useful.

High stable endemic type: This includes old hyperendemic areas with an estimated current API of > 5 and less than 10. Major part of north-east Delhi comes under this category. In Jumna riverine belt malaria situation has changed over a period of time. In the present study, API increased progressively from south to north.

Control strategy was suggested based on stratification of malaria and vector density in each area. In areas towards south-east of Delhi which were exposed to the risk of moderate malaria (< 5 API), control strategy suggested is to be directed against the aquatic stages using environmental management methods and wherever possible, biological control measures such as bacterial, larvicide spray, release of larvivorous fishes and putting oil at breeding sites, can be applied.

Receptive types: This includes all areas with estimated annual parasite index (API) below 5. Major part of north-west Delhi and part of south Delhi come under this category. All localities of this area are exposed to the expected risk of moderate to high quantum of malaria transmission.

In these areas control measures suggested are mainly engineering methods such as proper water gradient in cemented drains etc. and in developmental areas which were endemic for malaria, spraying of residual insecticides in the months of April-May and August-September should be done. Legislative measures should also be invoked for prevention of man-made malaria.

Epidemiological types and stratification could be used to develop appropriate, cost-effective and sustainable malaria control strategy.

P. vivax Relapse Pattern in North-East Delhi

P. vivax positive cases reported in MRC clinic from July 1988 to December 1993 were analyzed for relapse pattern. Cases reported between July and December 1988 were followed upto next 5 years. While all cases reporting between January 1989 and December 1992 were followed upto December 1993. All *P. vivax* cases were treated with 900 mg chloroquine base (adult dose) only.

A total of 2493 *P. vivax* patients reported in the clinic. Total number of episodes were 3340. A total of 724 (29.04%) patients relapsed with vivax infection during the follow-up period while the

remaining 1769 (70.96%) did not relapse. Among the total relapsed, maximum cases (61%) relapsed once, while the maximum number of relapses in a single case were 13.

Of all relapses 79% occurred within 1 year of follow-up, 90.3% occurred within 2 years and 95.3% cases in 3 years. Although the follow-up period in different cases ranged from 1 to 5 years, the above data indicates that 3 years follow-up was good enough as it could cover approximately 95% relapses. Similarly, cases relapsing upto 3 times accounted for 93.8% of total relapses.

Cases of primary attack registered from January to June relapsed within a short interval, whereas those registered from July to December relapsed usually after longer interval. Further, though January and Feburary are non-transmission winter months, there was a peak in primary attacks during this period, thereby indicating that these cases would possibly be due to a vivax strain with a long incubation period. The above data also suggest the presence of at least two different strains of *P. vivax* in north-east Delhi.

Epidemiological studies of urban malaria : Gujarat as a case study

Due to wide scale industrialization – construction of housing and commercial complexes, establishment of new townships and urban colonies is the present day scenario throughout the country. It has been observed that in most of the situations poor planning of health programme has resulted in manifold increase in disease incidence and in particular malaria incidence. Gujarat being one of the major commercial/industrial centre with high incidence of malaria has been selected for an epidemiological analysis of urban malaria. The following observations have been made in the study.

Gujarat is a peninsula state. Apart from several Aravalli off-shoots, there are important hills in the centre of the state giving rise to an almost perfect radial drainage system. The drainage of southern Gujarat as a whole is characterized by parallel rivers flowing directly into the sea. Negligible slope near the coast leads to seasonal water-logging and marshy conditions. Gujarat enjoys pre-dominantly tropical monsoon climate with significantly higher rainfall in the south-eastern areas which declines towards north-west. The Saurashtra peninsula is semi-arid with scanty rainfall.

Table 57 shows occurrence of local maxima and minima of the API curve where 'x' denotes maxima and 'o' minima. It also re-

Table 57. Cyclic pattern of urban malaria in Gujarat

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91
Ahmedabad	x	o		x			o		x	o	x		o	x		o
Amreli	x		o		x	o				x		o		x		o
Banaskantha	x				o		x					o				x
Baroda	x							o	x	o				x		o
Bharuch	x				x							o	x			o
Bhavnagar	x											o			x	o
Gandhi Nagar	x	o	x	x		o		x				o		x		
Jam Nagar	x			o		x	x					o		x		o
Junagarh	x		o				x					o			x	o
Kheda	x		o			x				o				x		o
Kutch	x			o				x			o			x		o
Mehsana	x		o			x		o	x		o				x	o
Panchmahal	x		o	x	o			x					o	x		o
Rajkot	x			o	x	o	x					o		x		o
Surat	x		o						x		o				x	o
Surender Nagar	x			o	x		o	x				o		x		o
Valsad	o	x	o	x			o	x		o				x		o

veals that in general API after resurgence in 1976 was high in 1989 and in between 1976 and 1989 high API cluster around 1983. Thus, it may be concluded that a 6-7 year malaria cycle is followed in the state.

Figs. 23a and b show trend of malaria cases per square kilometre of Gujarat as a whole from 1978 to 1984 and 1985 to 1991. In the first block of seven years 1978-84 the trend is slightly declining, whereas in the second block 1985-91, it is sharply increasing exhibiting manifold increase in density of malaria cases per sq km.

Figs. 24a and b show districtwise urban malaria trend in Gujarat for the period 1978-84 and 1985-91 respectively. From districtwise trend it is observed that in the first seven year block, 1978-84 out of 17 (2 districts in Gujarat namely Sabarkantha and Dangs being entirely rural) only 8 districts show increasing trend whereas in the second seven year block, 1985-91, 13 districts show an increasing trend of malaria cases/sq km. which shows that besides eight districts, five more districts are showing exclusively increasing trend.

Thus from both the Figs 23 and 24 an increase in malaria potential is observed throughout the state. The plains of Gujarat consist of districts Ahmedabad, Gandhi Nagar, part of Kheda, Mehsana, Surendra Nagar and Banaskantha. Ahmedabad and Gandhi Nagar are adjacent districts having similar ecological conditions. Gandhi Nagar district is very small in comparison to Ahmedabad in regards to population and geographical area, yet malaria incidence in Ahmedabad urban area is one third of Gandhi Nagar. However, the pattern of API over the successive years is similar. The vectors *An. culicifacies* and *An. stephensi* is reported to be resistant to DDT and HCH. High malaria in urban Gandhi Nagar may be attributed to high rate of migration (73%). In Ahmedabad, malaria shows a good correlation with rainfall.

The districts Amreli, Rajkot, Junagarh, Jam Nagar and Bhavnagar are under same ecological constraints and show a 4-5 year malaria cycle. Districts Jam Nagar, Rajkot and Junagarh have industries and class I towns. Specially in Jam Nagar, about 40% people work in industries. It appears that high malaria in these districts is due to large industrial setup. Amreli has two class I towns or big industrial set up but high migration rate to nearby areas may be the cause for increased malaria.

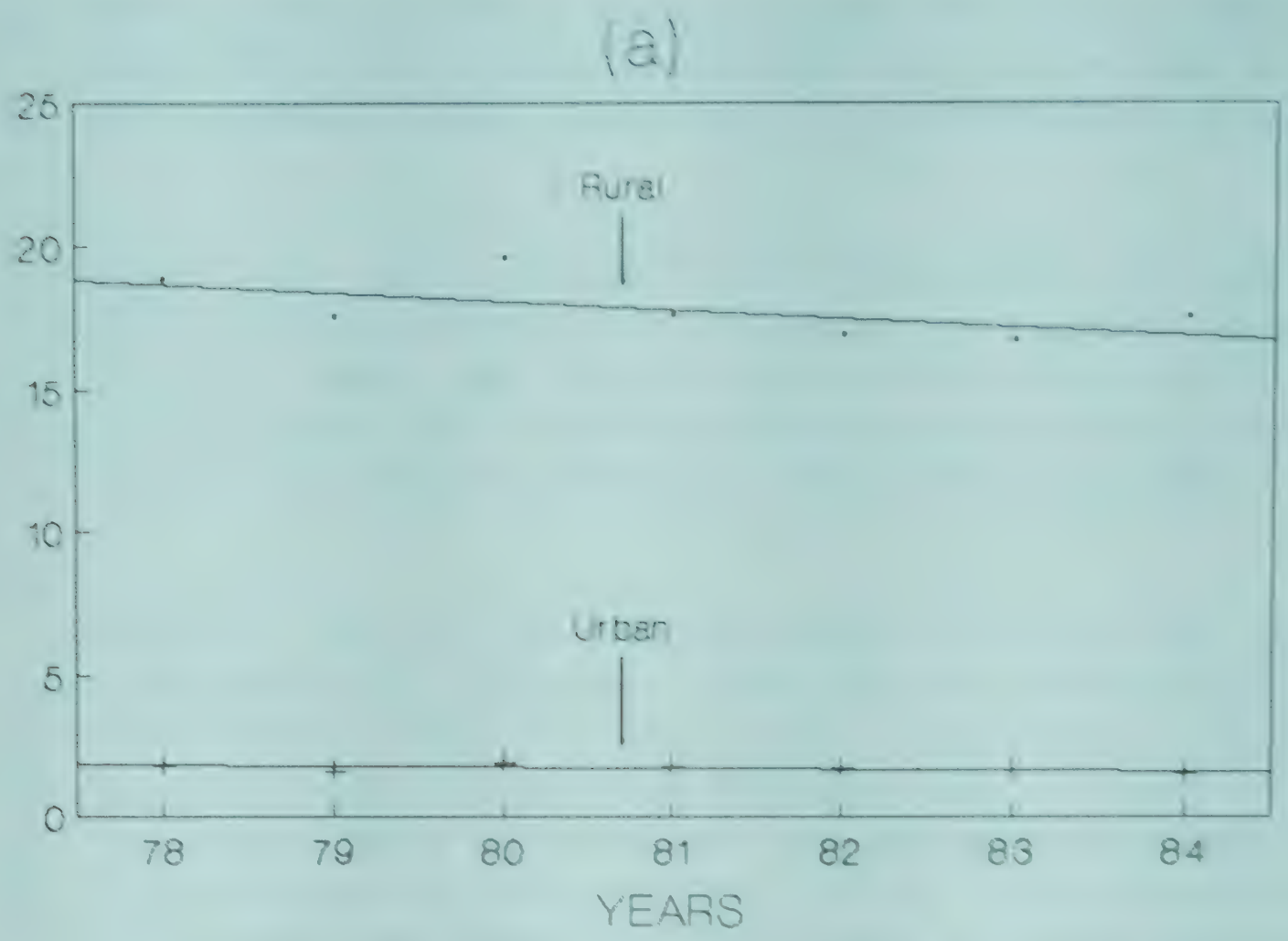


Fig. 23a: Trend of malaria cases per sq km in Gujarat (1978-84)

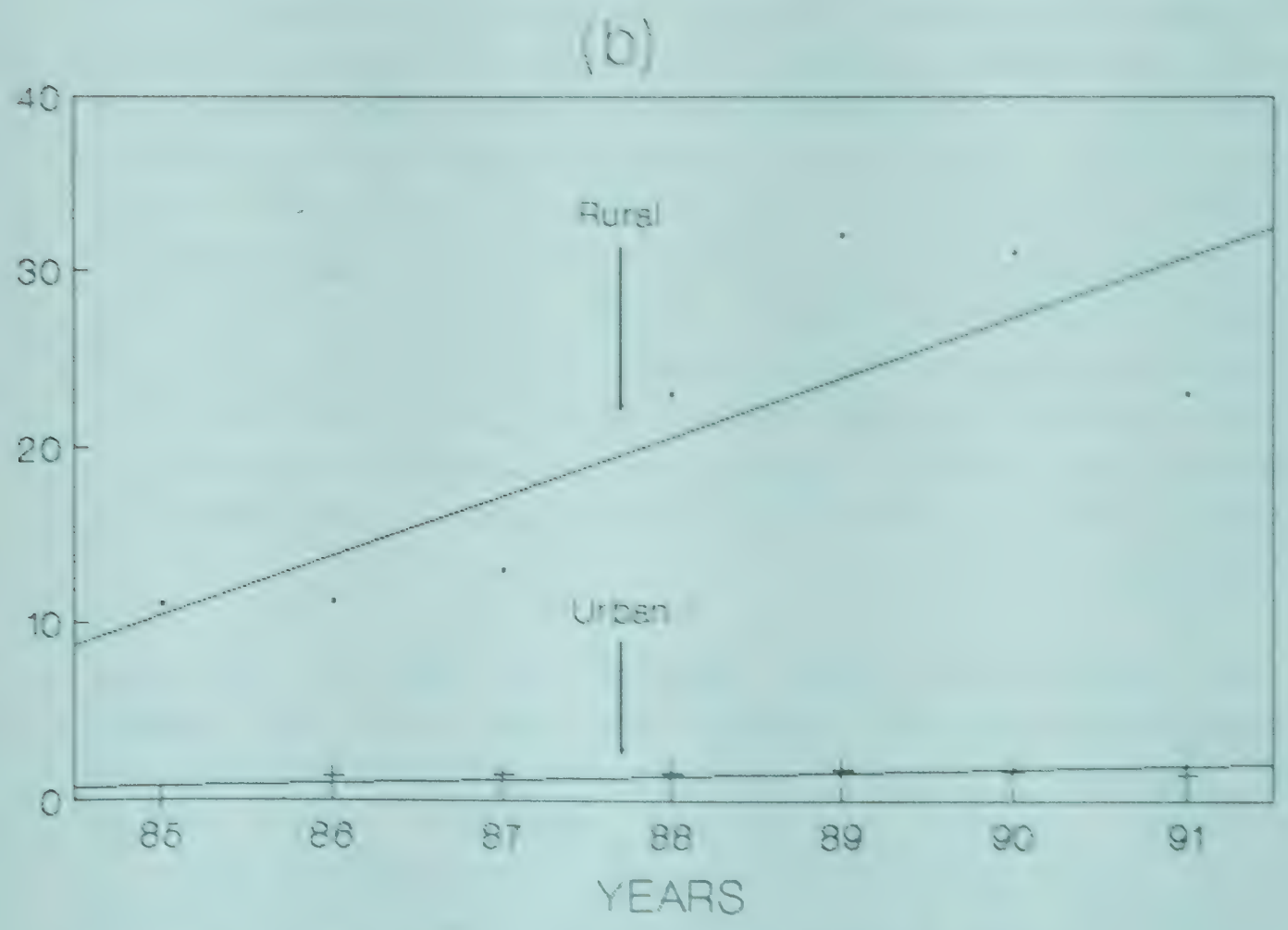


Fig. 23b: Trend of positive cases per sq km in Gujarat (1985-91)

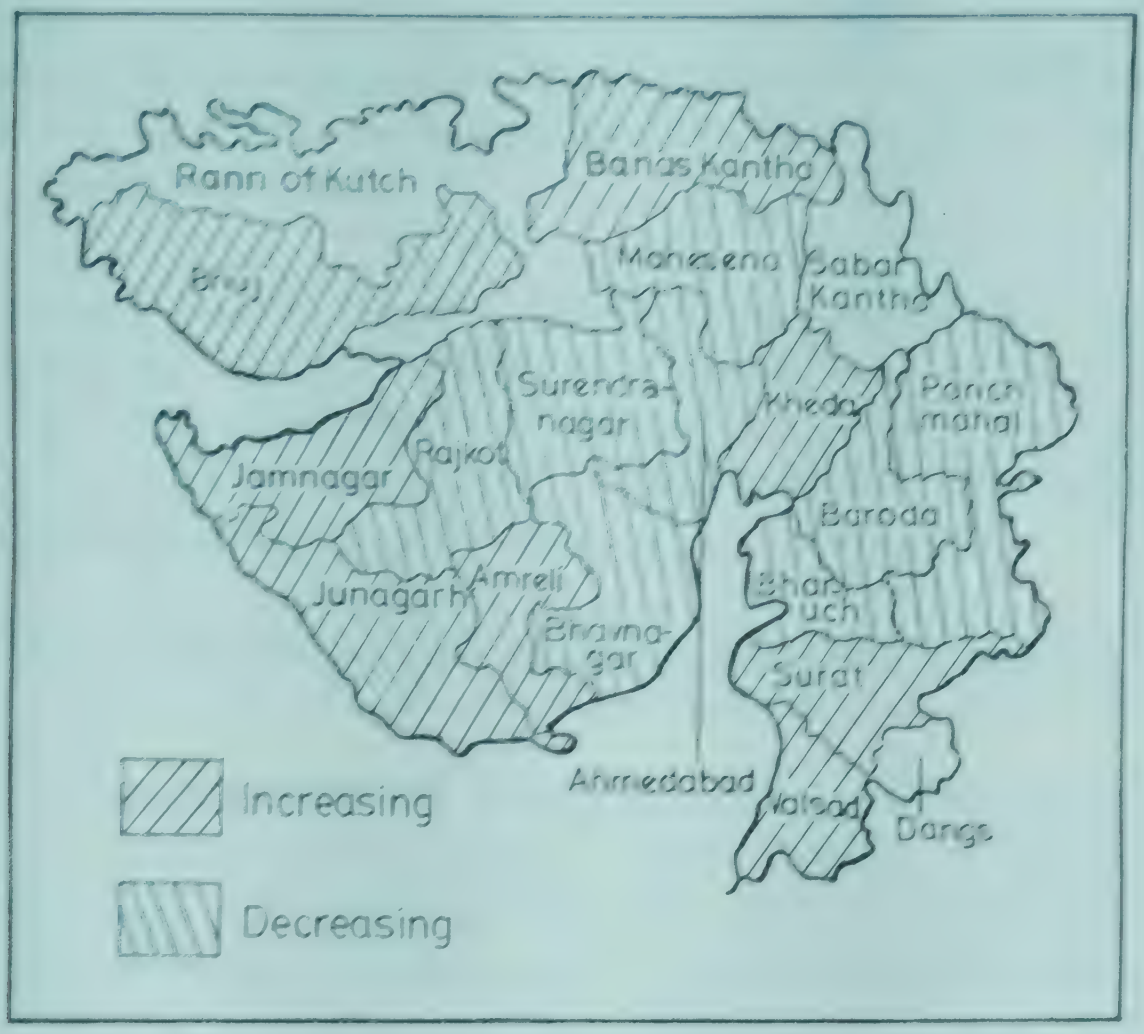


Fig. 24a: Urban malaria trend in Gujarat (1978-84)

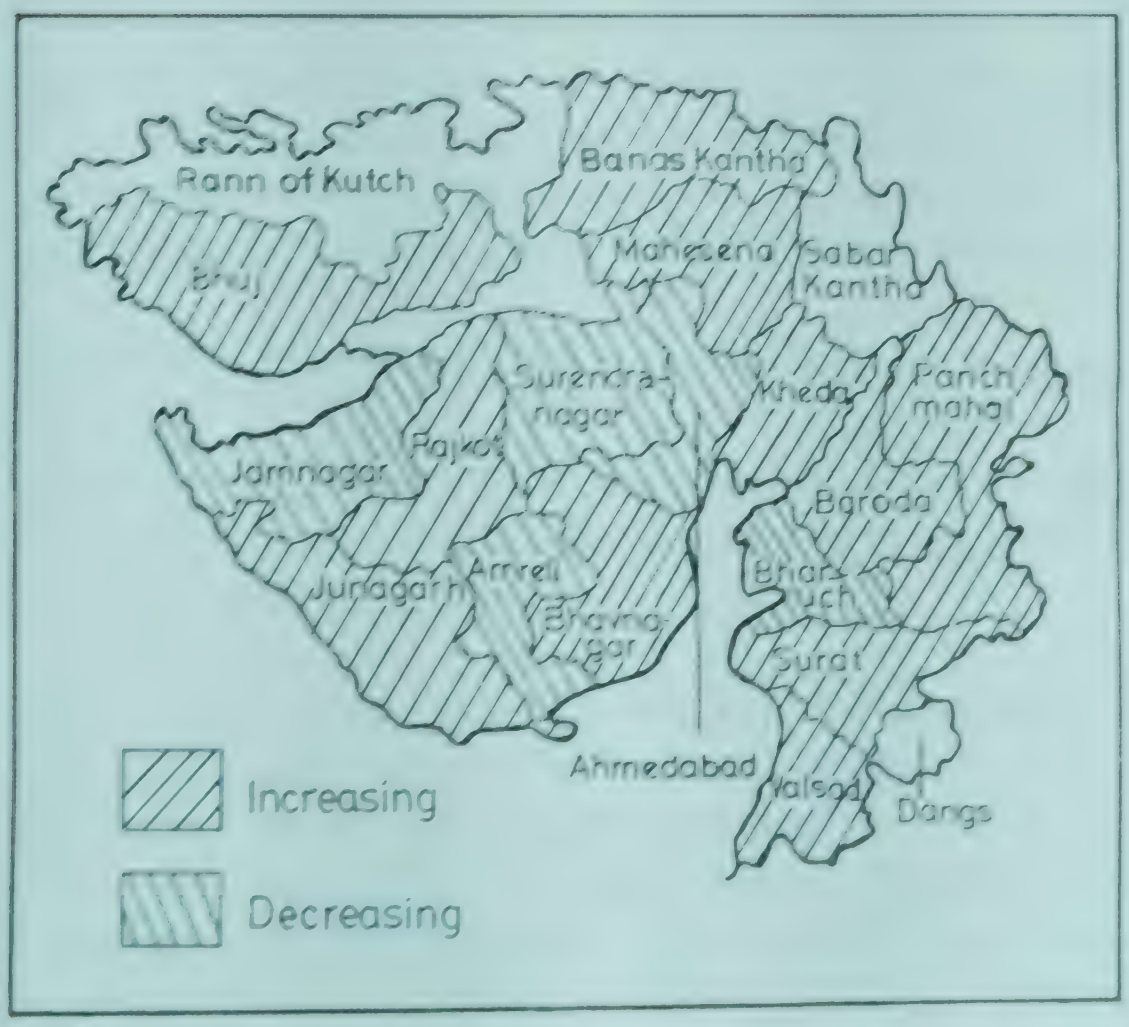


Fig. 24b: Urban malaria trend in Gujarat (1985-91)

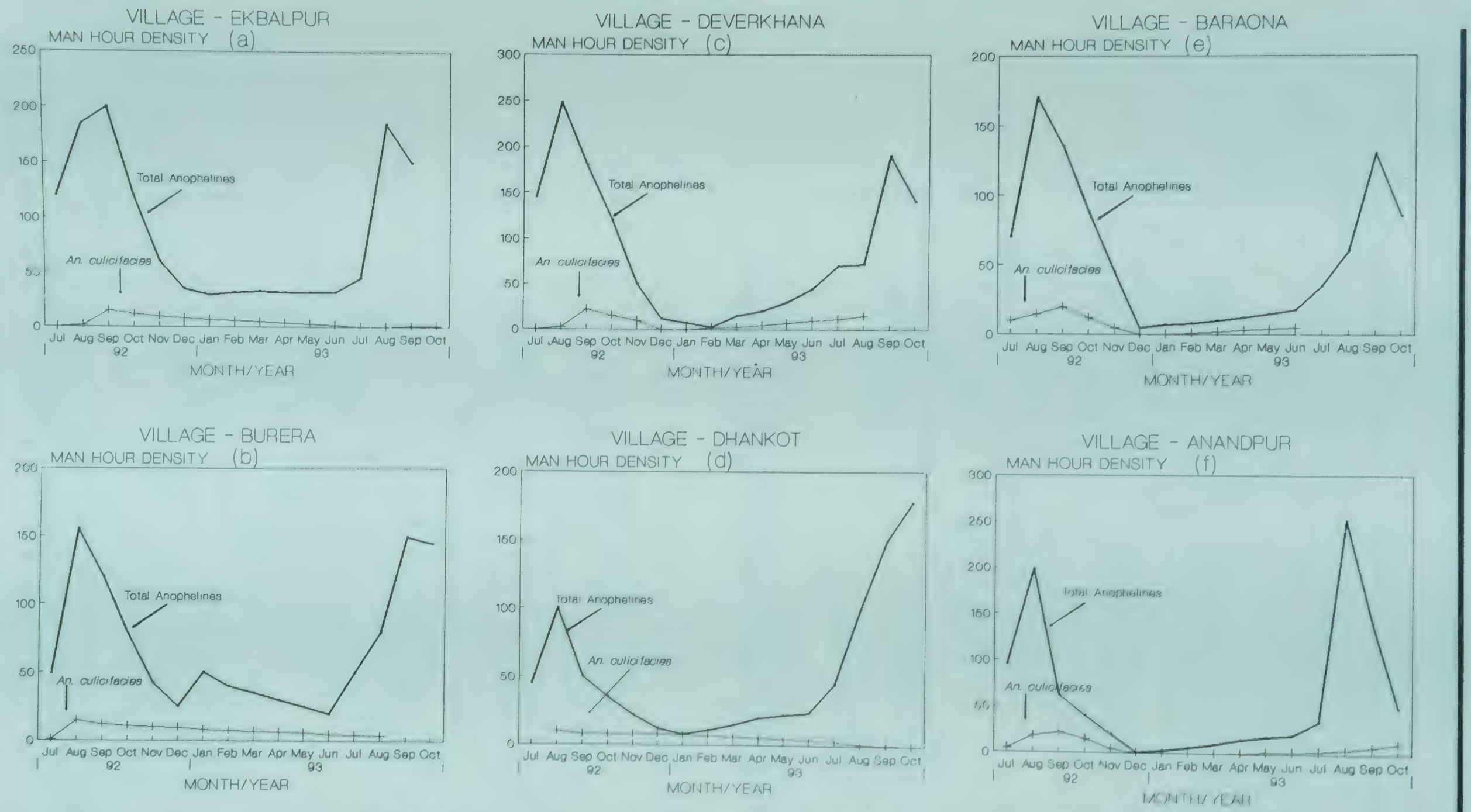


Fig. 25: Per man hour density of total *Anopheles* and *An. culicifacies*

The district of Kutch is characterised by negligible slopes and poor drainage. An increase is observed both in urban and rural areas. Malaria in Kutch is basically a rural problem. API in 1989 has been reported > 40 against an average of 20 in rural areas. The vector is highly resistant to DDT.

Topography of Surendra Nagar is typical as the general relief varies from below sea level to > 300m; it is saucer shaped with low-lying areas. In urban areas malaria is low as compared to rural areas. *Pf* % has shown increasing trend in both rural and urban areas but in urban areas increase is more sharp.

Impact of Gurgaon Water Supply Channel on Malaria Transmission

A study to see the impact on malaria transmission in the six selected villages situated on the bank of water channel in Haryana state was taken up. A detailed map has been prepared and baseline data on number of bridges, banking, non-banking, lining of the channel, water table etc. has been collected. Besides this, entomological data on adult and larval density is being monitored monthly. The man hour density of total anophelines *An. culicifacies* for 6 different villages are given in Fig. 25. The per man hour density of *An. culicifacies* varied from 0.5 to 18 in different seasons. The SPR of these 6 villages also varied from 7.8 in 1992 to 3.1 in 1993 respectively. The construction of canal is still in progress and water is expected to be released after monsoon this year which is bound to change malaria scenario in nearby villages. The study is in progress.

Vector-Parasite Interaction Studies on Salivary Gland Apyrase

A study was undertaken to acquire information about salivary gland apyrase levels in the uninfected and laboratory infected members of the *An. culicifacies* complex.

The salivary gland, a reservoir of infective stage of malarial parasite harbors highest concentration of an enzyme called apyrase. Salivary gland apyrase (SGA) is an important factor in determining the probing time of a vector on to the host. Infact, SGA facilitates the mosquito feeding process by efficiently disrupting the host platelet aggregation. The parasite is known to reduce SGA and compels the vector to visit more number of hosts. This situation can enhance the transmission potential of the vector.

Standardization of biochemical quantification of SGA is in progress. The information acquired through these investigations can give an insight into the mechanism of vector-parasite interactions and the dynamics of transmission.

Seroepidemiology of Malaria

Application of Serology to Estimate Malaria Endemicity

214 finger prick blood samples on filter paper were collected from Ekta camp of Vasant Kunj in June 1992. In this camp labourers from different states migrate for construction work carried out by the DDA. Local medical practitioners claim, this to be a highly malariogenic area. Under these conditions, we selected this area for a serological survey (Fig. 26). The antibody was estimated against *Pf* antigen and we found malaria transmission in the area. In each age group the antibody response ranged between 0.6 to 0.9 O.D. value.

Out of samples collected in July 1993, 15 were *Pv* positive 1 *Pf* and 31 negative. Synthetic peptide RESA (R1) and *Pf* (Fig. 27) was found to be R1 and gave high antibody response compared to *Pf* antigen (Fig. 27). Uniformly, the titre was found out to be moderate in all the age groups. It can be said that this camp had experienced moderate malaria transmission throughout the year.

Serological testing

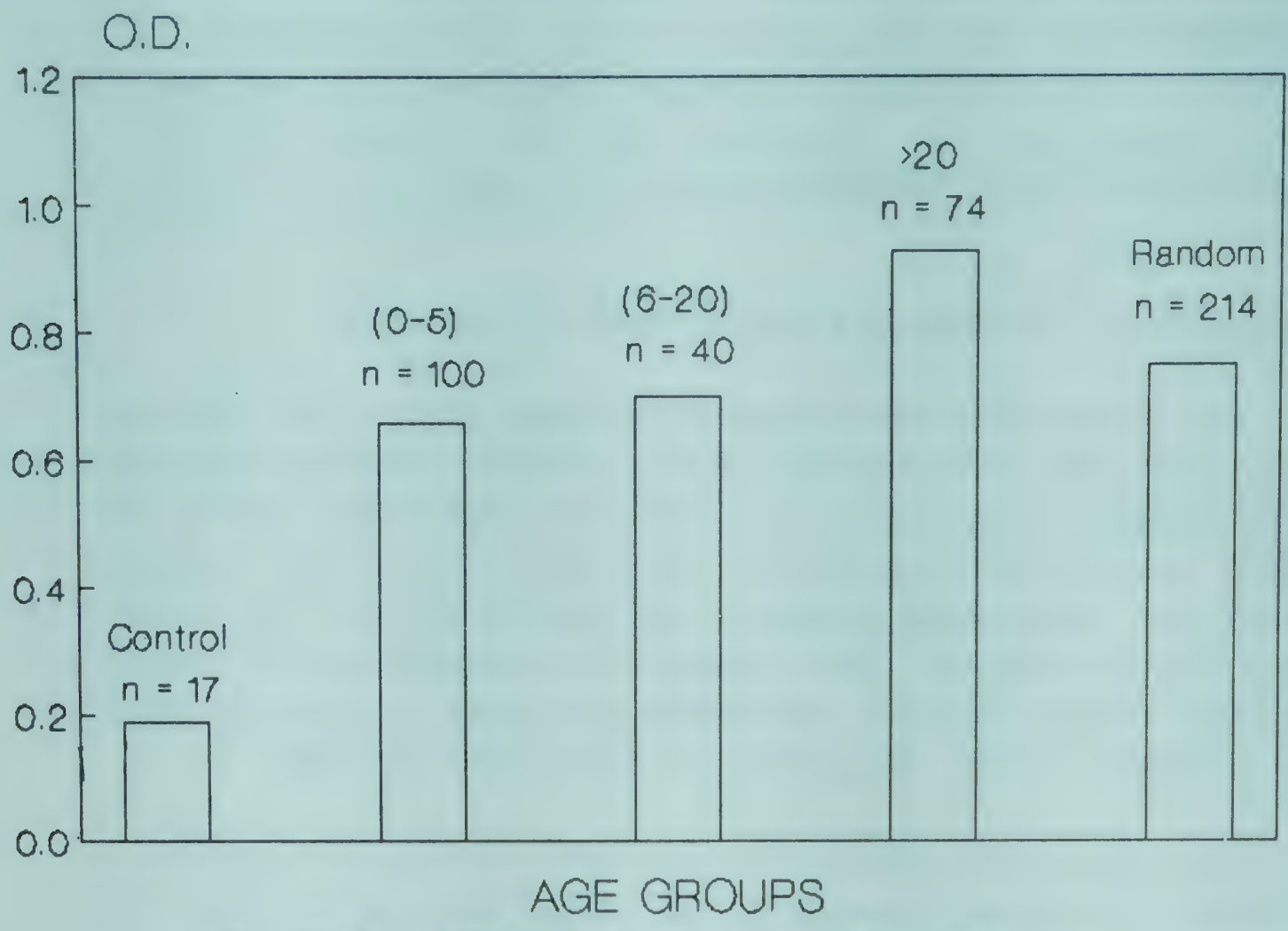


Fig. 26: Mean *Pf* antibody response of donors from all age groups in Ekta camp of Vasant Kunj, June 92

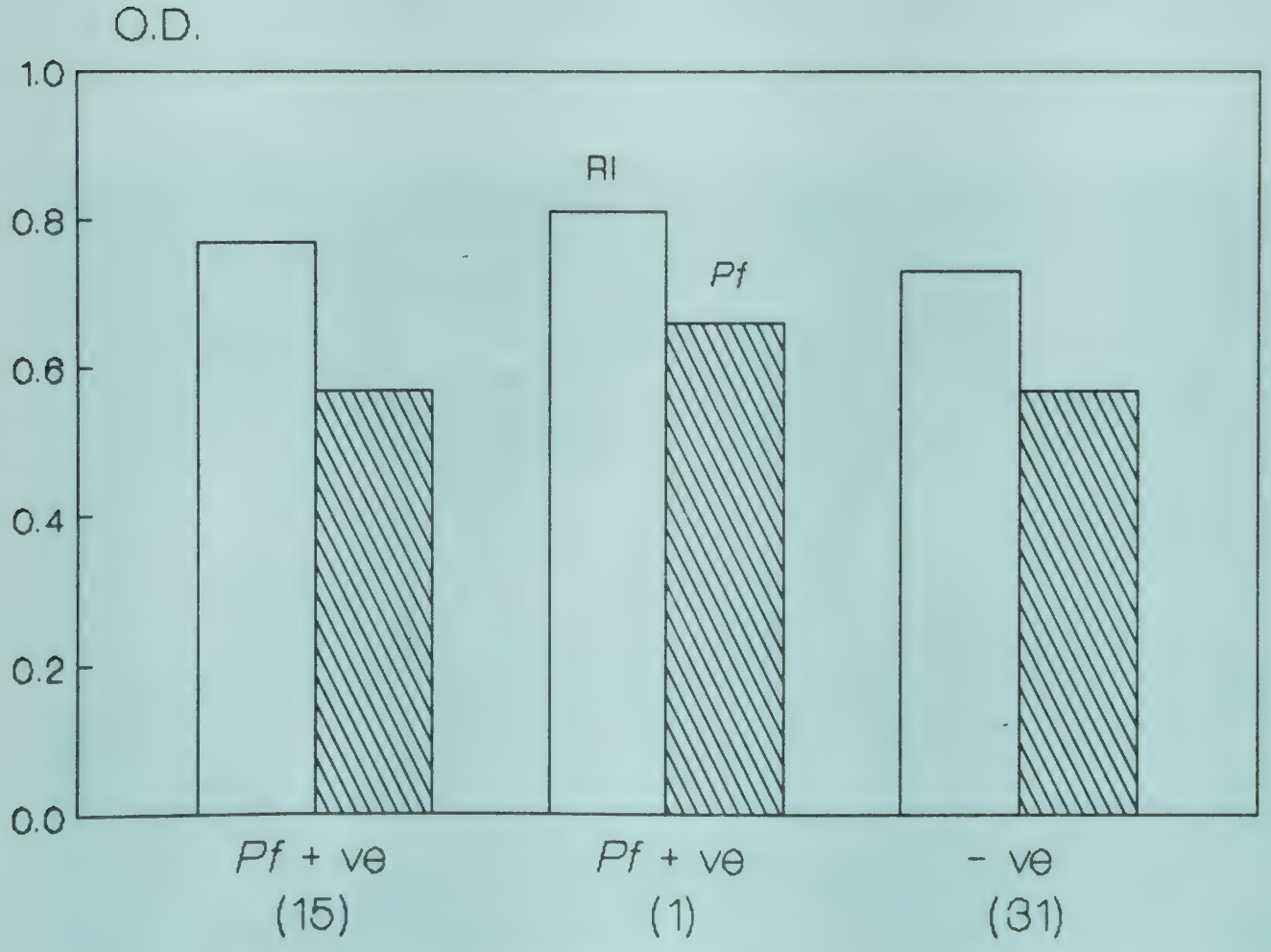


Fig. 27: Mean antibody response against RI and *Pf* antigen in malaria positive and control population of Vasant Kunj area, July 93

In order to study malaria endemicity, samples from Madras were collected. Since malaria is a local and focal disease, the variation in antibody response is very wide. During January 1993, 106 samples were collected. Antigen used was R1 (synthetic RESA peptide), salivary gland (SG) and *Pv*. In children and adults, the antibody response in Ramanathapuram was very high, indicating active malaria transmission (Fig 28). In Vedhalai HSC of Madras, the antibody response against all the antigens was much higher than in Ramanthapuram population, indicating a higher transmission (Fig 29). Whereas mean O.D. values of Mandapam, antibody response is lower in adult and children (Fig 30) indicating less incidence of malaria. Control population was taken from Delhi laboratory staff. Through serology it is possible to identify areas which are having low, moderate or high incidence of malaria according to their mean antibody response.

Determination of Cross-reactivity of *Pf, Pv* and SG with Non-malaria Patients blood Sample

We have collected 26 sera and filter paper blood sample of non-malarious patients from OPD section of All India Institute of Medi-

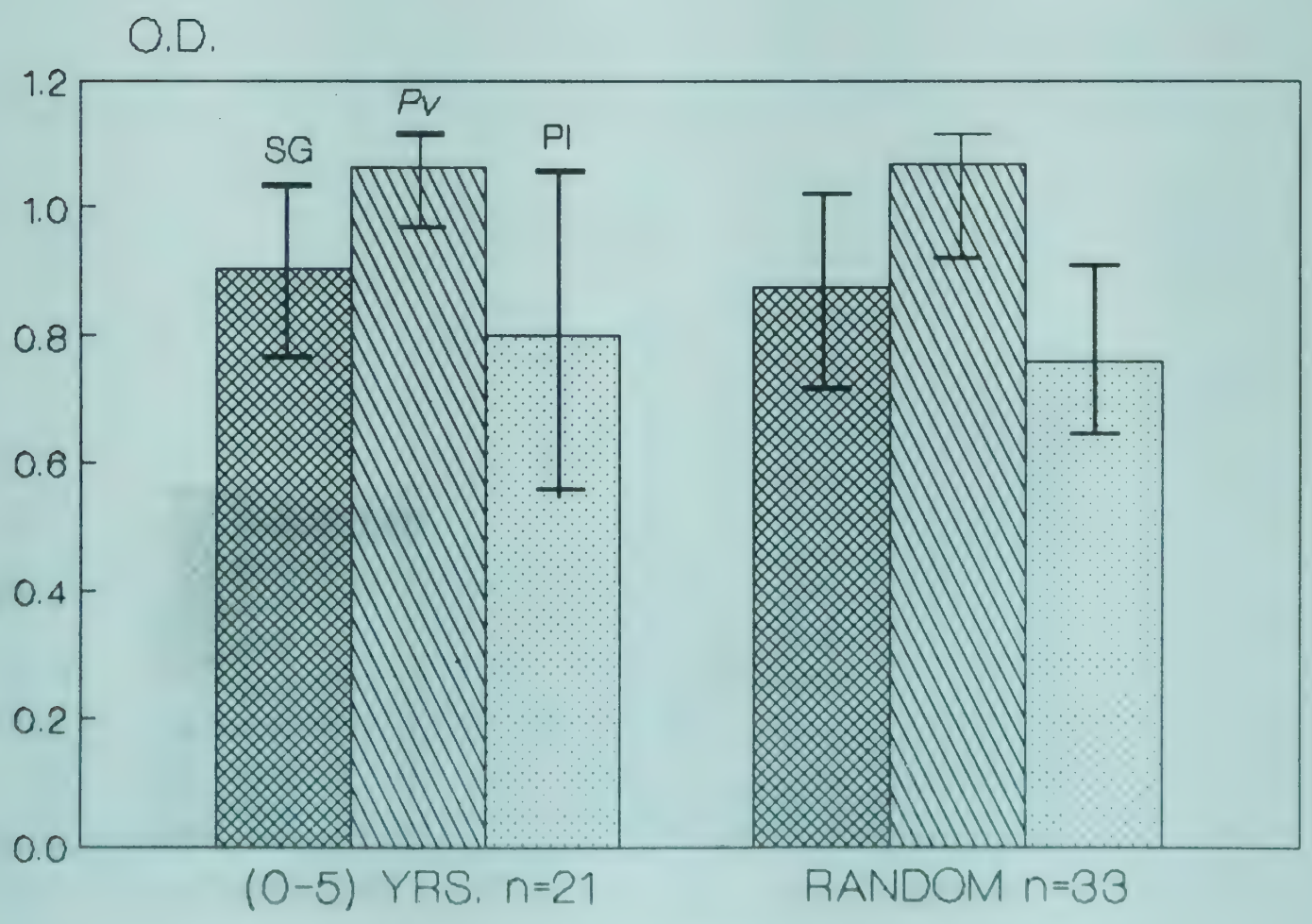


Fig. 28: Valayaravadi code 0902 PHC Mandapam District Ramanathapuram

COMMUNITY HEALTH CELL
LIBRARY AND DOCUMENTATION UNIT
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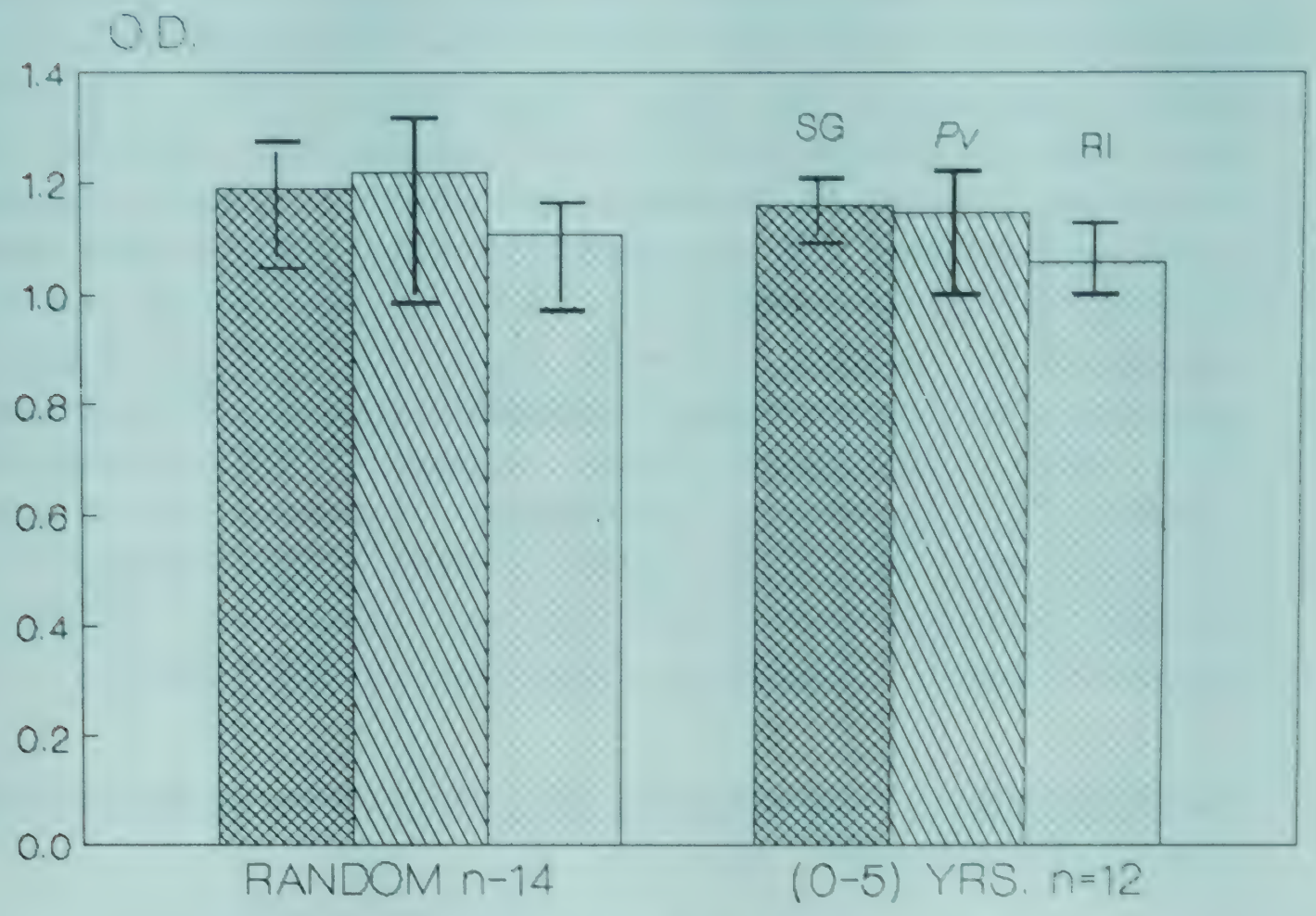


Fig. 29: Antibody response against SG, *Pv* and R1 among adult and children in Vedhalai HSC of Madras

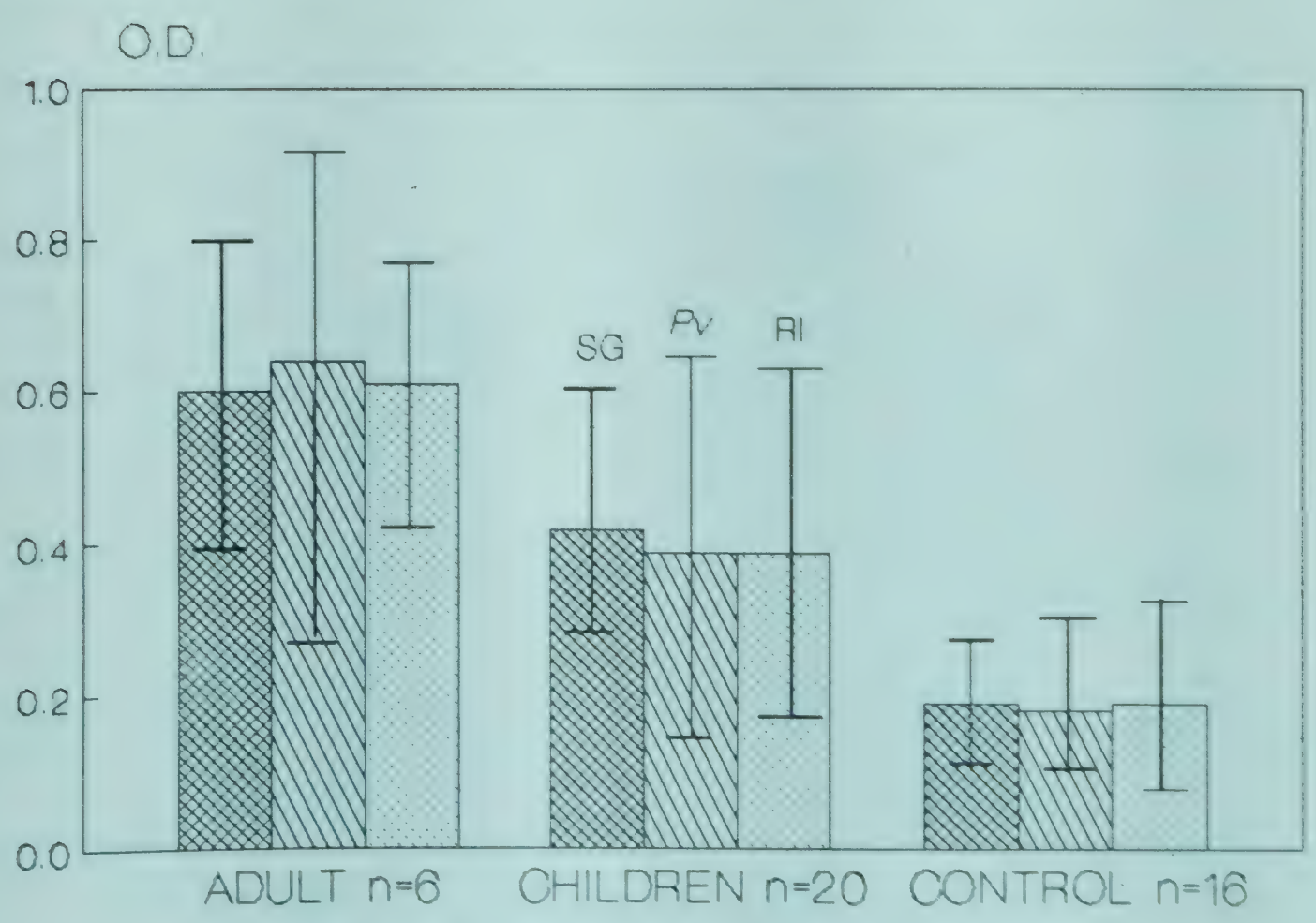


Fig. 30: Mean antibody values of Mandapam camp among adult, children and control population

cal Sciences, New Delhi. The sera were collected from patients having *E. Coli*, Kala-azar, Toxoplasma, TB and Rheumatoid arthritis infection. This was done to see whether there is any cross-reactivity between antibodies with *Pv, Pf* and mosquito salivary gland antigens (SG). Non-malarial patients were found to cross-react with all the antigens (Fig. 31). The response is low to moderate. Correlation between serum and filter paper was studied (Fig 32) and found to be good. Filter paper eluate gives higher antibody response than serum. The importance of this study is that today blood eluate gives higher antibody response than serum. The importance of this study is that today blood eluate antibody has been accepted widely to collect finger prick blood in filter paper as it is convenient. But in comparison with the filter paper, serum corelates much better with the clinical manifestation. Three different dilutions were used. At 1:100, difference between serum and blood was much less than 1:1000 dilution. The antibody could be segregated broadly in 2 zones. One is low antibody response which has O.D. value < 0.25 and moderate > 0.25. Our study indicates antibody response of non-malarial patients was lower compared to malaria patients.

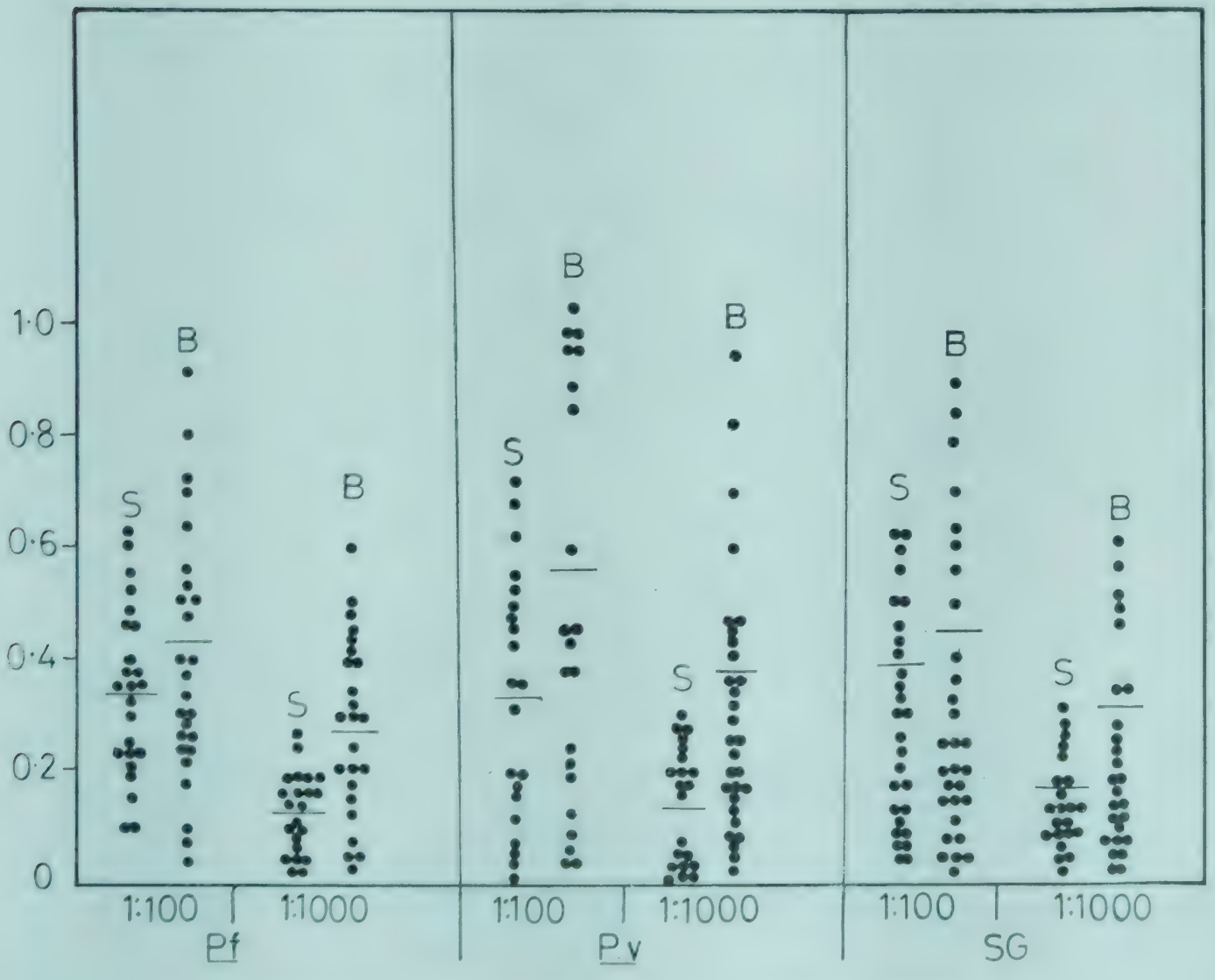


Fig. 31: Comparison of antibody response in individual donors using serum (S) and filter paper (B)

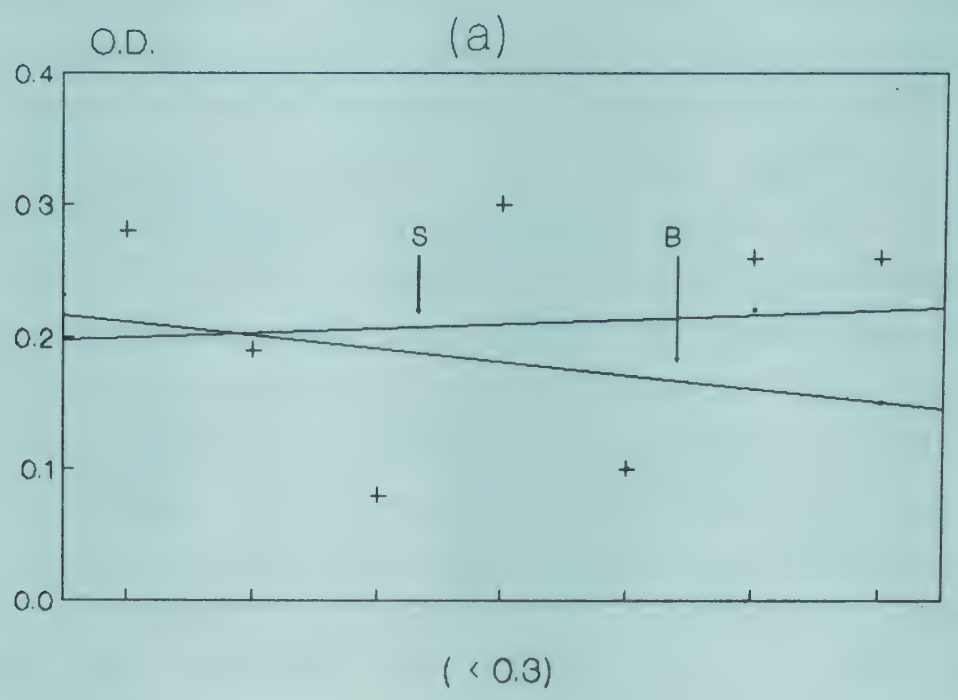


Fig. 32: Correlation of serum and filter paper blood O.D values with *Pf* antigen

Identification of Antigenic Components

Culture supernatant of *Pf*, FSJ strain was taken for the study. It contains many soluble antigenic components, which are released during schizogony. It also contains 10% human sera. In order to separate out antigens as glycoprotein from the crude mixture it was fractionated. Prior to fractionation 500 ml culture supernatant was concentrated, dialysed and lyophilized. Crude culture supernatant which was in the lyophilized form was used for fractionation. It was passed through antihuman sera affinity column and culture supernatant lyophilized product was again passed through the column and 2 fractions were identified. 1st major peak was identified as glycoprotein (Fig 33). It will be further characterised. This antigen will be used for looking at the protective antibody.

Immuno-diagnostic Tests for the Detection of Plasmodial Antigens by Enzyme-immunoassay

Diagnosis is required adequately for infectious diseases to attribute a successful treatment. The use of immunological methods for diag-

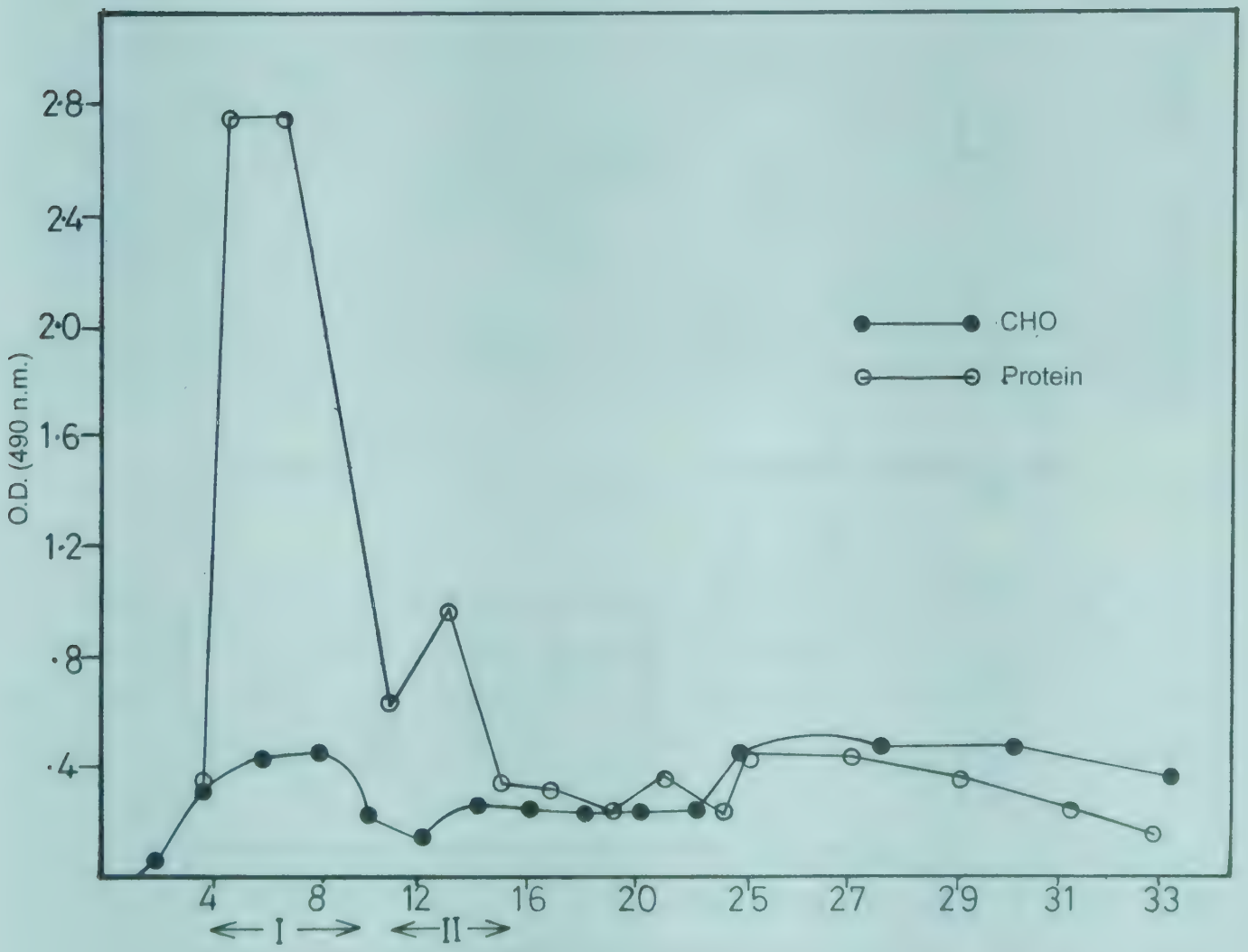


Fig. 33: Fractionation of culture supernatant (P.P) FSJ strain

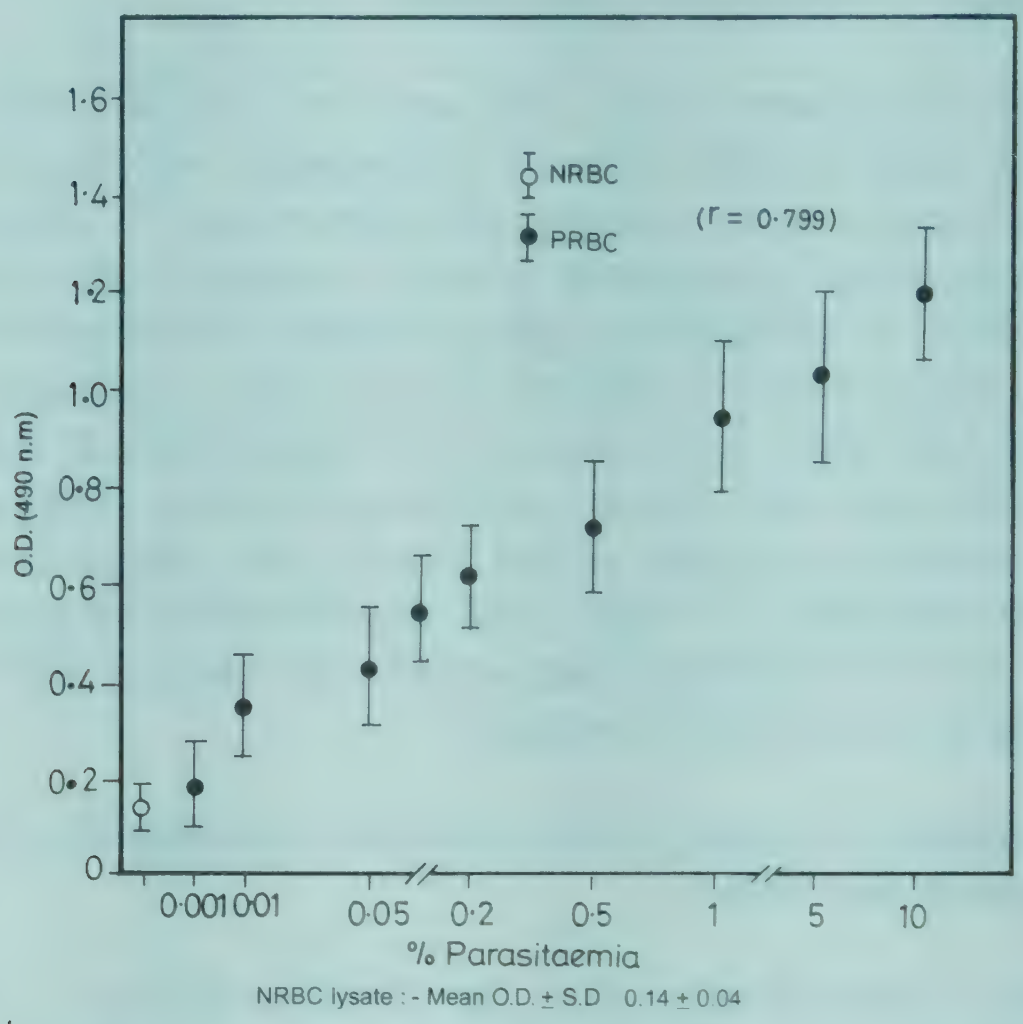


Fig. 34: Sensitivity of Sandwich ELISA for detection of parasites

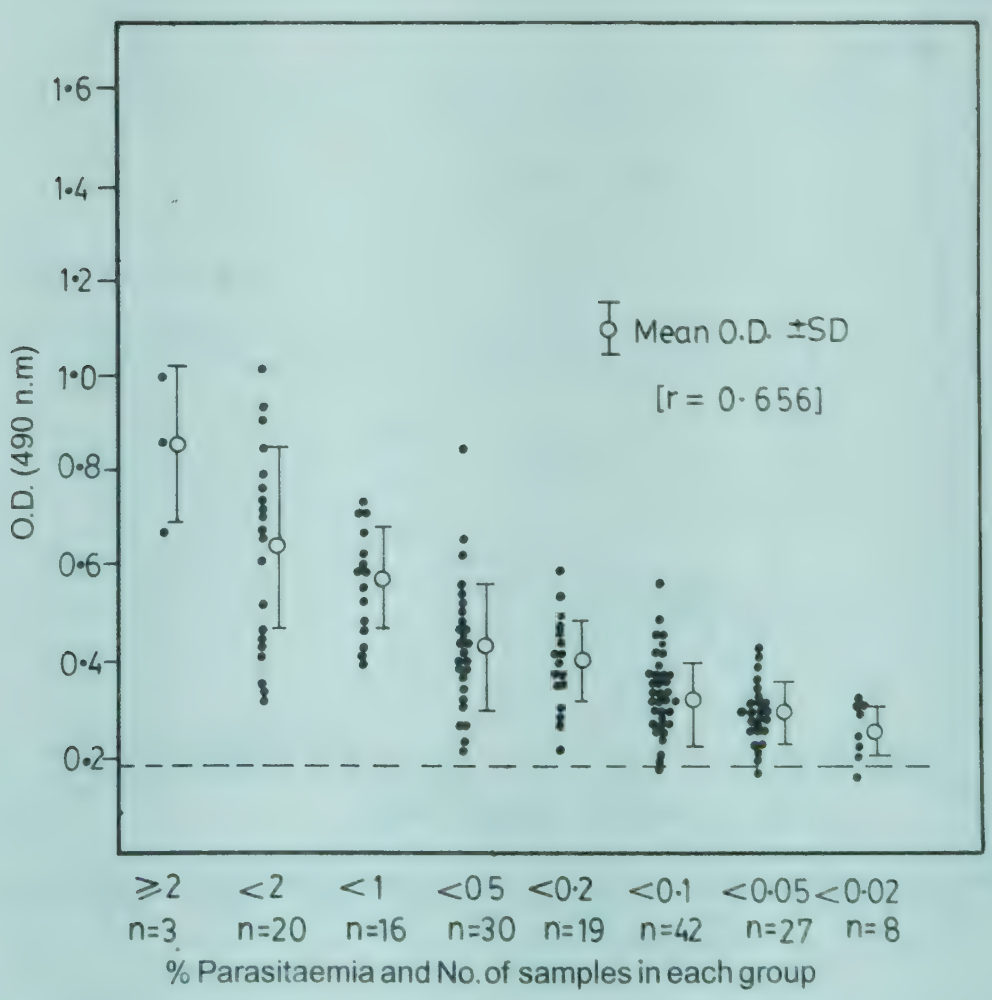


Fig. 35: Comparison between groups of *P. falciparum* patients with EIA

nostic purposes may help in the detection of ongoing or recent infection, disease prevalence and infection dynamics.

In continuation with earlier studies, the polyclonal anti *P. falciparum* antibody raised in albino mouse was used to detect presence of parasites in patient's blood. This antiserum was purified by salt precipitation and by affinity chromatography. Human sera with high antimalarial antibody titre (above 1/512) were pooled and then purified. Purity of these antibodies were tested by immuno-precipitation technique. Sandwich ELISA method was adopted for parasite detection due to its simplicity and small requirement of immuno-reagents than Inhibition ELISA.

ELISA technique in progress in immunology division

Polystyrene microtitre plates were coated with 100 µl of mouse anti Pf-IgG (100 µl/ml). After blocking with BSA, parasitized and normal RBC lysates were added at serial dilution in triplicate and incubated. Antibody antigen complex was trapped by purified human immune IgG by adding in each well (50 µg/ml). Sensitivity of Sandwich ELISA was compared with per cent parasitaemia and non-specific reaction with normal RBC lysate was taken as cut-off point (Fig. 34). This test was applied in a group of individuals with *P. vivax* and *P. falciparum* malaria and also in patients with fever and slide negativity (Figs. 35, 36). The assay showed diagnostic sensitivity at parasitaemia above 0.01%. Since, it was a polyclonal antibody assay,

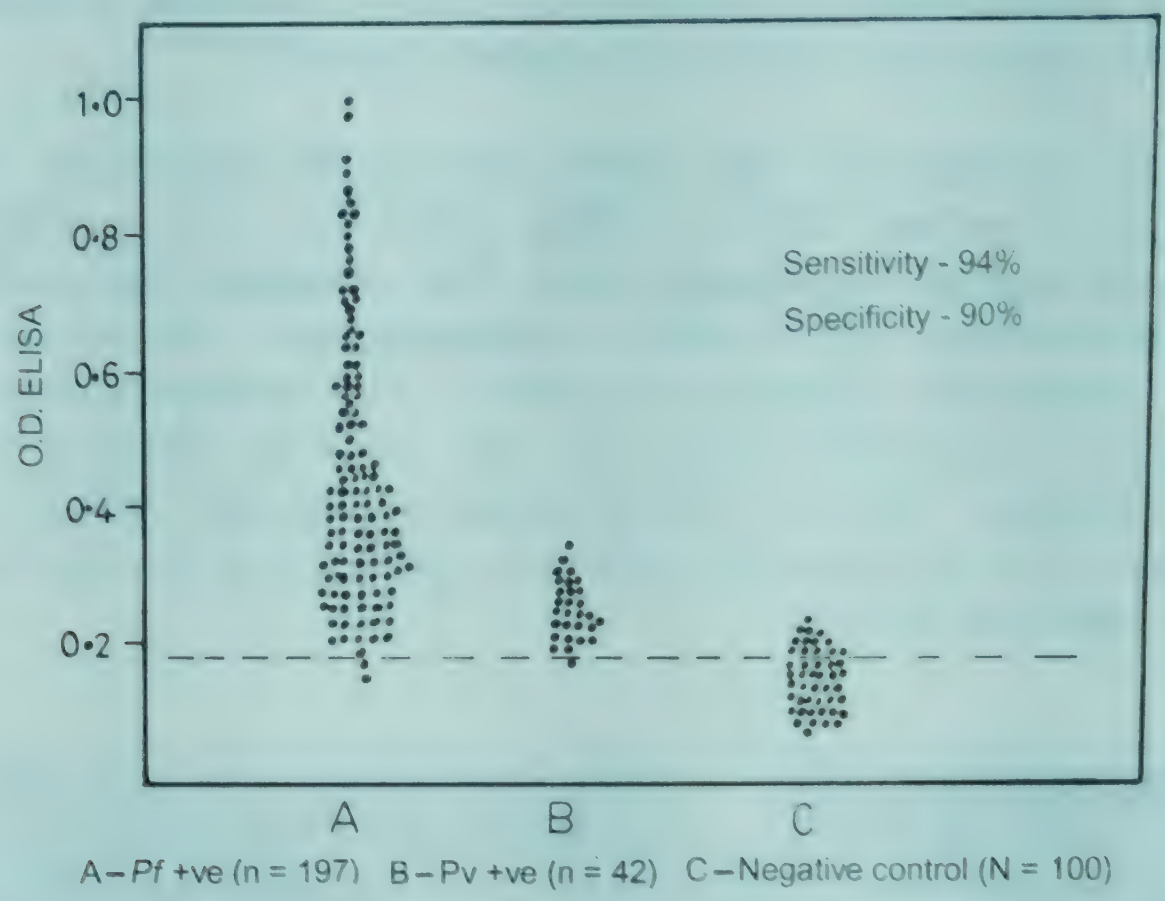
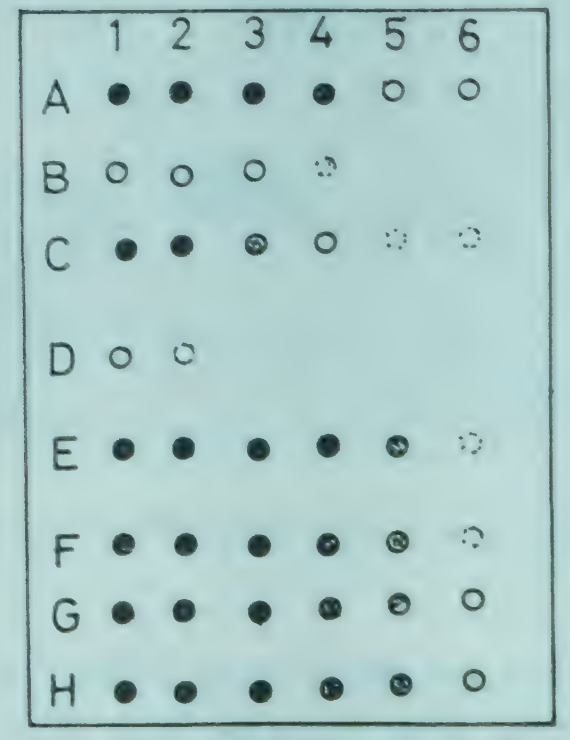


Fig. 36: Scattering pattern of samples tested for antigen detection by enzyme immunoassay



Nitrocellulose membrane was treated with anti-*Pf* antibody. Parasitized-RBC and normal RBC lysates were spotted on antibody coated membrane. Human malaria immune IgG was used as second antibody. Patient's blood having parasitaemia at the range of 2% to 0.001% was used (lane: A,B,C,D,E,F,G,H) as positive and normal RBC lysate (O+) and MP negative blood (lane:B,D) was used a known negative.

Fig 37: Microdot ELISA test for *Pf* antigen detection

it detected *P. vivax* parasites also. A Micro-dot blot ELISA was also developed in antibody coated nitrocellulose membrane which showed a little higher sensitivity than Plate ELISA (Fig. 37). Studies are in progress to test the reproducibility, sensitivity, specificity and time requirements.

Factors Involved in the Spread of Chloroquine Resistant Plasmodial Infections

Different mechanisms are responsible for evolution and spread of chloroquine resistance (cr) of *P. falciparum*. However, none of them can fully explain the fast propagation of resistant foci. The introduction of chloroquine resistance *Pf* is possible even in areas without undue use of chloroquine. There are several hypotheses proposed. One of them was the involvement of one or more genes. In endemic areas, the two factors affecting parasite growth and population would then be host protective immunity and chemotherapy. Epidemiologically this is reflected as drug pressure and herd immunity.

Nothing is known about involvement of factors in induction of chloroquine resistant *P. falciparum* (CRPF) in India. The estimation of CRPF in different areas of India and study of multifactorial factors like immunity, nutrition, misuses of chemotherapy will give basic concepts of *P. falciparum* ecosystem in India.

In order to understand the nature of immune responses during an infection with chloroquine resistant and susceptible malaria parasite infection, we have studied the rodent parasite *P. berghei* in BALB/c mice. Three groups of mice, five in each (1) control, (2) mice infected with cr and (3) sensitive to chloroquine (CS) *P. berghei* were included. Two groups of mice were infected with resistant and susceptible parasites and kinetics of parasitaemia (different stages) were monitored till the last day of survival. The infected groups were sacrificed when the parasitaemia reached 10% and control were also sacrificed on the same day. Spleen cell were prepared and proliferation was measured after stimulation with or without antigens after five days by thymidine incorporation. Supernatants were collected for IFN-gamma estimation before thymidine pulsing. Crude lystate of both resistant and sensitive *P. berghei* antigens were used for this assay. Antibody production *in vitro* by these cells in culture supernatants in response to these antigens were also determined by ELISA after stimulation for 12 days. Malaria specific antibodies from filter paper elutes

of all mice before and after infection at 10% parasitaemia level were measured by ELISA against both resistant and sensitive crude parasite lysates.

Mortality occurred earlier in resistant infections (Fig. 38). Gametocyte conversion rate was more in chloroquine susceptible infections (Fig. 38). Both the antigens could induce spleen cells from sensitive *P. berghei* primed mice to proliferate and to secrete malaria specific antibodies *in vitro*. However, *in vitro* cellular and humoral responses with spleen cells obtained from chloroquine resistant *P. berghei* primed mice was lower in comparison with the susceptible infections (Fig. 39). The differences were significant between control and infected mice and also between infections with susceptible and resistant *P. berghei* parasites. Specific immunosuppression is an established phenomenon in malaria infections. Our studies suggest, that immunosuppression to parasite derived antigen is predominantly seen in chloroquine resistant infections in rodent models. Further studies are in progress to confirm these findings in the mice models and also to investigate the immune responses in the humans in cr and cs *Pf* infections.

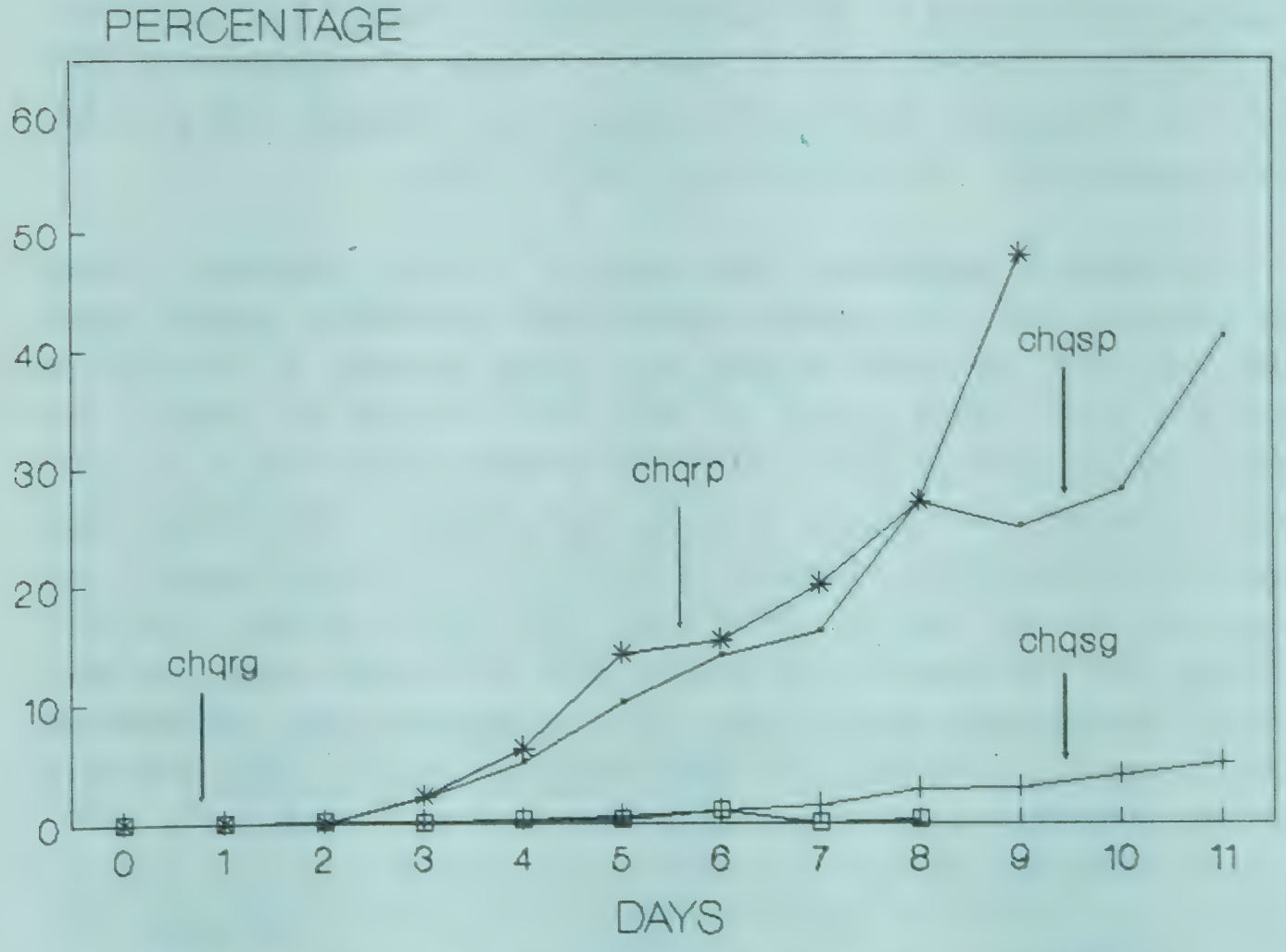


Fig 38: Chqr and Chqs P.B infections (Parasitaemia and gametocytemia)

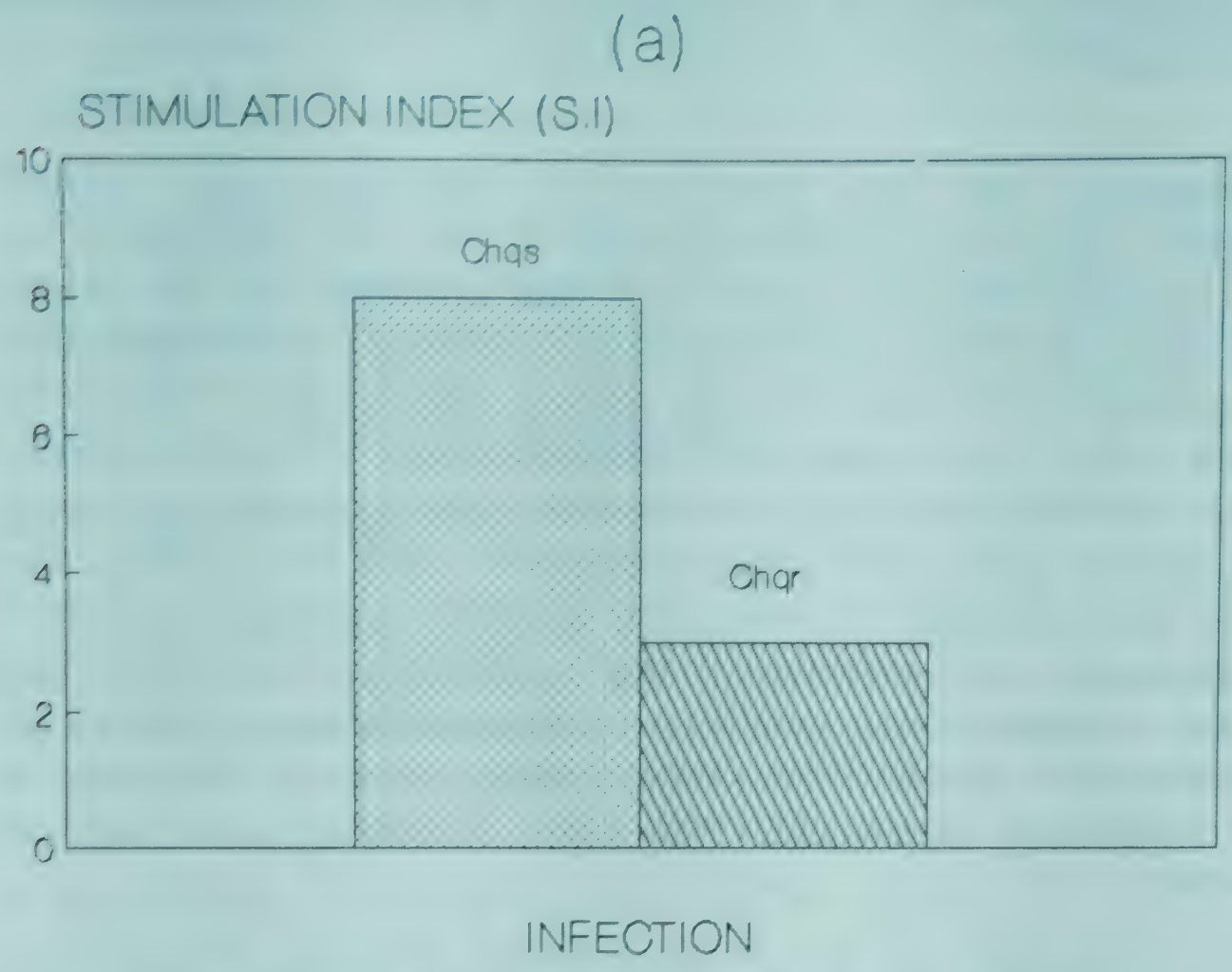


Fig. 39a: Chqr and Chqs P.B infections (T cell responses)

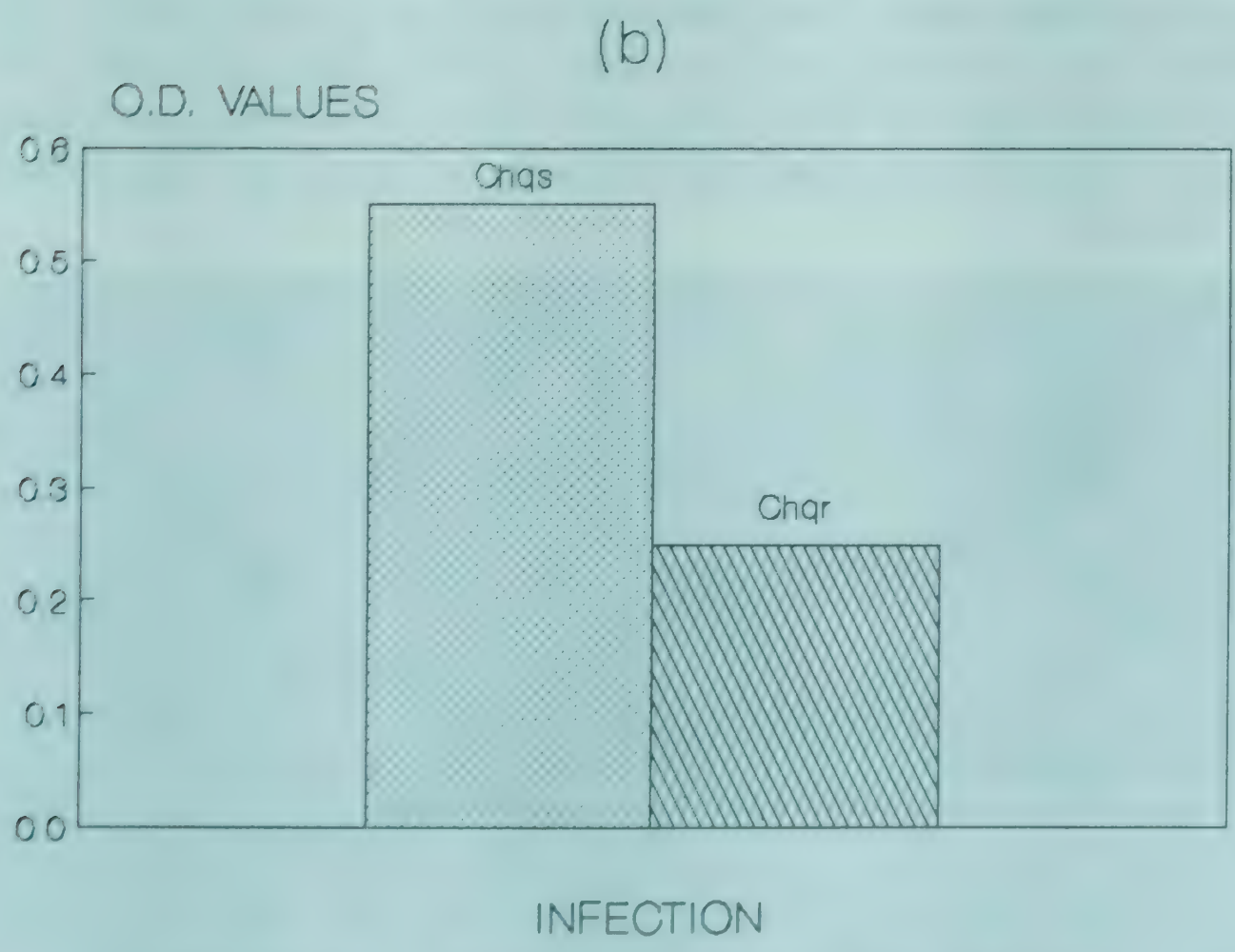


Fig. 39b: Chqr and Chqs P.B infections (B cell responses)

Production of Monoclonal Antibodies Against *P. vivax* Circum Sporozoite

Techniques have been standardized for the maintenance, cryopreservation and revival of myeloma cell line, SP_2. The technique of fusing antibody producing spleen cells with myeloma cell line (Kohler and Milestein, 1975) was followed for producing monoclonals against sporozoites of *P. vivax*. For this purpose BALB/c mice were immunized with *P. vivax* sporozoites by direct biting of infected mosquitoes. Sporozoite positivity in mosquitoes was confirmed by examining salivary glands of few mosquitoes from each batch before using them for immunizing the mice. Anti-sporozoite antibody was detected in the serum of immunized mice after 3 fortnightly immunization doses during a period of one month. Sporozoite harbouring mosquitoes and SP_2 myeloma cell lines are being maintained in our laboratory for this purpose. A booster dose was given 24 h before sacrificing the animal.

After confirming the presence of specific anti-sporozoite antibody in the mouse serum by immunofluorescent technique, the spleen was removed aseptically for harvesting the spleenocytes. Spleen cells from sporozoite immunized mice were fused with myeloma cell SP_2; this time the fusing agent being polyethylene glycol (M.W. 1500). The cultures were screened for clones after 14 days; few clones were detected. The clones developed (Fig. 40) were sub-cultured supernatants positive for anti-sporozoite antibody were collected, and kept frozen for analysis.

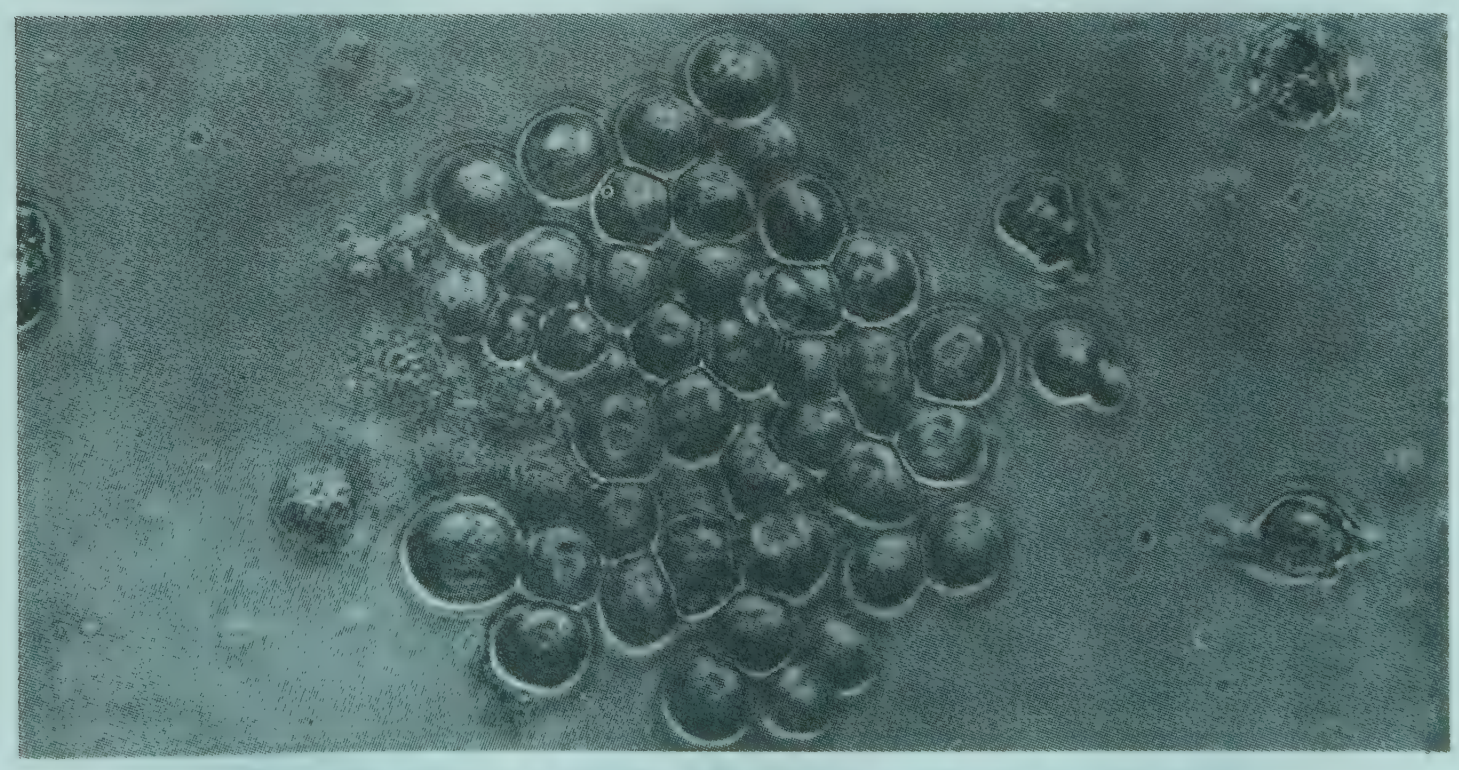


Fig. 40: Anti-sporozoite (*P. vivax*) antibody producing clone, *in vitro*

SCIENCE AND TECHNOLOGY PROJECT ON THE INTEGRATED VECTOR CONTROL OF MALARIA, FILARIA AND OTHER VECTOR-BORNE DISEASES

During 1993 S&T project has made important contributions in regard to the development an field testing of new technologies. In certain area NMEP and state health departments collaborated with the project staff in the transfer of technologies. Major achievements of the S&T project are briefly· describe below.

Field Trials with Biolarvicides

Bacillus thuringiensis H-14 and *Bacillus sphaericus*, the two biolarvicides were imported from the former USSR (now Russia) for trials on the control of breeding of vectors and non-vectors in a variety of water-bodies in endemic areas of the country. Laboratory evaluations were carried out at the MRC headquarters and field testing was done at 12 locations in the country. In about 6 months it was clearly demonstrated that biolarvicides provide effective and extended control of mosquito breeding. A document titled ''Progress Report of Biolarvicides'' was published by the Centre. This report provides details of field trials and results of related studies/observations. Trials with biolarvicides have been planned for 2 years. Results of another 8 months are similar as was observed in the first 6-months. Acute toxicity data show safety to non-target species and warm-blooded animals. Results are consistent with the expectation that biolarvicides can replace chemicals in the control of mosquito breeding.

Studies on the Mosquito Breeding in Rice-agroecosystem

Rice-fields produce enormous mosquito populations. Studies were therefore directed to see the potential of rice-fields in the production of malaria vectors. A two year study was initiated at Hardwar, Haldwani, Mandla and Rourkela field stations. Studies at all the field stations have shown that breeding of malaria vectors e.g., *An. culicifacies* is insignificant or non-existent in most areas. *Anopheles* breeding is affected by the agronomic practices and irrigation. In some areas fallow fields are more dangerous than the rice fields. This study will continue for another year to enable us to draw the final conclusions.

Neem in Malaria Control

Low concentrations of neem oil were found to repel mosquitoes and sandflies. A 2-4% neem oil in coconut/mustard oil was tested at

various field stations. It was revealed that (i) neem oil was an excellent repellent; (ii) Anophelines are repelled better than Culicines, and (iii) repellent action on sandflies is more pronounced than mosquitoes. Neem oil can also be used on the mats or in kerosene lamps. Experiments have also started on the larvicidal action of neem oil. Initial experiments have shown promise both in the repellent action of neem oil and killing effect on the mosquito larvae.

Workshops on Transfer of Technology to the States

Several workshops on the (i) engineering methods of malaria control and (ii) management of serious and complicated malaria cases were organized with the help of field stations. These workshops were very useful in introducting new technologies and in bringing awareness.

Highlights of Specific Work at each Field Station

1. **Karnataka State:** A unit of MRC is functioning satisfactorily in Bangalore with the specific task unit to transfer the technology of the bioenvironmental malaria control methods to the state health department. The work has started in Kolar district which contributes 36% of the total and 46% of *Pf* cases reported from the state annually. There are 60 PHCs in Kolar district. Geographical reconnaissance (GR) has been completed in Kamasamudram, Budikota and Sadali PHCs. GR of Gudibanda is in progress. Detailed maps of these PHC's showing problem villages, mosquito breeding sites and proposed intervention methods have been prepared for implementation by the health department. State health staff was also given training and larvivorous fishes are being mass produced. Studies on species composition of the malaria vector *An. culicifacies* have shown the presence of species A (vector species) in sufficient numbers.

2. **Madras (Tamil Nadu):** MRC is working in close collaboration with the Municipal Corporation of Madras in extending the bioenvironmental control of malaria to the entire town. Overhead water tanks, wells and sumps in the government building are being mosquito proofed on priority basis. Inspection of OHTs and wells in the private colonies is in progress and more notices have been served. MRC is maintaining complete control of mosquito breeding in 3 corporation divisions comprising 90,000 population. In addition to this, studies in progress are (i) field trials with the biolarvicides; (ii) tests on the larvivorous fish poten-

tial; (iii) studies on the biology of *An. stephensi*; (iv) studies on the breeding of mosquitoes in rice-fields; (v) relapse pattern in *P. vivax* (vi) chloroquine-resistance in *P. falciparum*, and (vii) mosquito breeding in sewage farm. Results of these studies would be available in 1994 for publication.

3. **Delhi State:** Master action plan to control mosquitoes and malaria in Delhi has been submitted to the state health department and Sanjay Gandhi Foundation, Delhi. Action plan brings out major mosquito production areas, endemicity to malaria and intervention strategies. To control mosquito breeding, biolarvicides have been tested. A larvivorous fish farm is being maintained. Repellent action of neem oil and control of mosquito breeding with neem based larvicides are currently under test at the MRC.

4. **Panjim (Goa):** Bioenvironmental interventions by MRC successfully suppressed malaria epidemic and brought down malaria cases from 5-6 thousand to 422 in 1992. At this point, the strategy was passed on to the health department. After withdrawal by MRC, cases in 1993 increased to 1411. This clearly brought out that there was need for improvement in the quality of health services. In this area a major programme was to demonstrate the impact of biocide spraying on malaria transmission and reduction in mosquito densities. Spraying of biolarvicides reduced mosquito breeding. As a result impact of spraying was visible in the reduction of adult densities and the transmission of malaria.

5. **Hardwar (U.P.):** Bioenvironmental control interventions at the BHEL and IDPL are in the maintainence phase. The study was extended to IOC, Mathura, and bioenvironmental malaria control reduced transmission in the IOC campus. Surveys in other industrial areas showed high transmission and excessive consumption of anti-malarials. Spraying of biolarvicides at the BHEL reduced mosquito breeding. *B. thuringiensis* H-14 was effective in the control of *Ae. aegypti*. Monitoring of insecticide residues revealed high contamination of water bodies with the chlorinated hydrocarbon insecticides. Methods have been developed to monitor the plasma levels of anti-malarials in human blood.

6. **Sonapur (Assam):** Epidemiological studies revealed outbreaks of malaria in many areas. SPR was 29% and *Pf* per cent 79%. *An. minimus* is the vector which is often found in high densities maintaining stable malaria. Field station is promoting the use of insecticide impregnated bednets and health education for early diagnosis and full treatment. Repellent action of neem oil was

tested on *An. minimus* and results revealed about 90% protection from *An. minimus* bites. Studies have been initiated on the sibling species complex in *An. minimus* and *An. fluviatilis*. Workshops on the management of serious and complicated malaria cases were organized in tea gardens.

7. **Car Nicobar (A & N Island):** Bioenvironmental interventions have substantially reduced malaria in Car-Nicobar Island. At the time of launching of the strategy API was 140 in 1989 which has been reduced to 16.71. *Gambusia* fish has been released in *An. sundaicus* breeding habitats and it has proved very effective in the control of mosquito breeding. Installation of one way sluice gates have reduced breeding of *An. sundaicus* to very low levels. Fish fauna of the Island has been surveyed and their biological control potential against mosquito larvae evaluated. Survey of filariasis showed high mf rate from 5 to 25% in some Islands. Further work on reduction of malaria transmisssion is in progress by installation of more sliuce gates, release of larvivorous fishes and early case detection and treatment. Biolarvicides (*Sphaericus*) have produced excellent control of *An. sundiacus* breeding in a variety of habitats.

8. **Haldwani (Nainital district) U.P.:** Role of rice land agro-ecosystem in malaria transmission was investigated. It was revealed that high *An. culicifacies* densities in April and May were related to the mosquito breeding in rice-fields. Succession of vectors in rice ecosystem was investigated, and it was revealed that *Culex vishnui* group of mosquito breed heavily in rice-fields. A focal outbreak of malaria in Gadarpur villages was investigated. Biocides were tested in various habitats to control mosquito breeding and these were found highly effective in controlling mosquito breeding. Malaria clinic maintained at the field station show high SPR (27.1%) and 11.1% SfR. Larvivorous fishes were mass produced, and these are being maintained in some areas.

9. **Nadiad (Kheda district) Gujarat:** Parasitological studies in Nadiad villages have shown that malaria cases are on rise. In *P. vivax* relapse rate is about 15% which goes down to 4.5% with 5 day radical treatment of primaquine. *P. falciparum* is becoming resistant to chloroquine and 5-6% cases show resistance. Resistance surveys were extended to other parts of Gujarat, and chloroquine resistance in *P. falciparum* is widespread. During the year studies were initiated on the malaria potential of Wanakbori weir on river Mahi in Kheda and Sardar Sarovar Project in Barauch district. Baseline entomological and epidemiological data

has been collected which show high malaria potential, studies on the action of neem oil in coconut/mustard oil had excellent repellent action lasting for 12 hours. Studies are also in progress on the discovery of new biological control agents. A larvivorous fish farm would be made functional in a few months.

10. **Shankargarh (Allahabad district), U.P.:** Parasitological surveys in stone quarries revealed high malaria transmission (SPR 30.3% and SfR 20.7%). At the malaria clinic based on 5270 slides examination SPR was 46.7% and SfR 28.8%. These results were substantiated by mass blood surveys and infant parasite rates which were high. Tests on the sensitivity of *P. vivax* to chloroquine with 10 tablets (150 mg each) showed that the local strain is fully sensitive as the parasitaemia did not appear within 28 days.

11. **Mandla (M.P.):** A hospital-based study revealed 52% malaria, mortality due to cerebral malaria cases (27 died out of 52). *P. vivax* infections were causing abortions, and these cases are being documented. Studies on *P. vivax* like parasite revealed possibility of the discovery of a new parasite. Studies on the new parasite are in progress at the molecular level. Base line data has been collected on the impact of Narmada project on the malariogenic potential. Several ecological methods were evaluated in the field to study vector biology and behaviour. Studies on rice agro-ecosystem revealed that malaria vectors do not breed in rice-fields. Neem oil produced good repellent effect on the mosquitoes.

12. **Shahjahanpur (U.P.):** The primary objective of this field station for a 2 year period is to demonstrate the impact of biocide application on the control of malaria in Farrukhabad district villages and on filariasis in Gursahaiganj villages. Spraying at fortnightly intervals of *Bacillus thuringiensis* and *Bacillus sphaericus* showed clearly the control of mosquito breeding, reduction in adult densities and impact on malaria transmission. Filariasis control was based on vector incrimination, and although there are less infective mosquitoes, the results are still inconclusive. A study of *Pf* sensitivity to chloroquine showed that the parasite is susceptible.

13. **Rourkela (Orissa):** Studies with pyrethroid impregnated bednets in mining areas and tribal villages showed that there was significant reduction in transmission of malaria. Studies on the rice-fields showed that rice-fields contribute poorly to malaria vectors but supported heavy breeding of *Culex vishnui* group of mos-

quitoes, which are vectors of *Japanese encephalitis*. Trials with biolarvicides reduced breeding of malaria vectors and non-vectors. Studies on the role of genetic polymorphism in malaria are in progress.

Other Highlights of the S&T Project

(i) Organized workshops on the engineering methods of malaria control.

(ii) Studies on the cost-effectiveness of various methods of malaria control were completed.

(iii) Several video films were prepared and telecasted on Doordarshan.

(iv) Work on remote sensing (RS) and geographical information system (GIS) was initiated. Nadiad taluka (Kheda district) and Car Nicobar Island have been taken-up for these studies.

(v) Environmental interventions were tested in the control of sandfly populations.

(vi) Health education and teaching materials were developed.

BIOLOGICAL MATERIAL BEING MAINTAINED AT THE CENTRE

Mosquito Strains

An. stephensi

Wild strains
Okhla, Delhi
Sarojini Nagar, Delhi
Phrazerpura, Maharashtra
Sonapur, Assam Goa
Nadiad, Gujarat

Morphological mutants
Red-eye (re) - sex-linked recessive
Black-larvae (Bl) - autosomal dominant
Golden-yellow (gy) - autosomal recessive

Biochemical variants
ODH-I, golden yellow
Me-S wild, Madras, T.N.
Me-F red eye
Got - F, Phrazerpura, Maharashtra
ODH-I, golden yellow - got fast
ODH-I, golden yellow - got slow
Jabalpur wild - got fast

Insecticide resistant/susceptible strains
Malathion resistant, Black-larva
Malathion resistant, wild, Sonepat, Haryana

***An. culicifacies* complex**

Species A

Wild strain, Ghaziabad, U.P.
Wild strain, Delhi

Species B

Wild strain, Shahjahanpur, U.P.
Wild strain, Ghaziabad, U.P.

Morphological mutants
Red-thorax (Rt) - autosomal dominant and creamish-larva (cr) autosomal recessive

Species C

Wild strain, Allahabad, U.P.

Species D

Wild strain, Jabalpur, M.P.

An. fluviatilis

Wild strains
Hardwar, U.P.
Haldwani, U.P.

An. annularis

Wild strains
Ghaziabad, U.P. and Shahjahanpur, U.P.
Sonepat, Haryana
Rourkela, Orissa

Culex quinquefasciatus

Wild strains
Ghaziabad, U.P.
Sonepat, Haryana
Delhi
Tetracycline treated aposymbiotic line

Insecticide resistant/susceptible strains
Sonepat fenitrothion resistant

Aedes aegypti

Wild strain, Delhi

Sandfly Strains

Phlebotomus papatasi
Ghaziabad
Maharashtra
Bihar

Phlebotomus argentipes
Bihar

Malaria Parasites

Simian Malaria Parasites
P. cynomolgi bastianelli
P. knowlesi
P. fragile

Avian Malaria Parasites
P. gallinaceum
P. relictum

Rodent Malaria Parasites
P. berghei NK-65
P. berghei (quinine resistant)
P. berghei (chloroquine resistant)
P. berghei NK-65
(Chloroquine sensitive, infective, gametocyte producing strain)
P. chabaudi
P. vinckei petteri 279
P. yoelii
P. yoelii nigeriensis (multi resistant)
P. yoelii yoelii 265
P. yoelii nigeriensis (infective, gametocyte producing strain)

Human Malaria Parasites
P. falciparum in vitro - 95 isolates adapted, cryopreserved and periodically revived
Chloroquine susceptible - 40 isolates collected from different areas-adapted and cryopreserved
Chloroquine resistant - 12 isolates collected from different areas
41 isolates not tested for chloroquine susceptibility

An infective gametocytes producing strain - NF-54
From CDC, Atlanta, GA
3D 7A, A clone of NF-54

Biological Control Agents

Fishes
Gambusia affinis – Delhi and Madras
Poecilia reticulata – Murad Nagar, U.P. and Bombay, Maharashtra
Puntius sophore – Murad Nagar, U.P.
Danio rerio – Rourkela, Orissa
Tilapia mossambica – Calcutta, W.B.

Notonectid bug
Enithares indica – Haldwani, U.P.

Nematode
Romanomermis iyengari – Pondicherry

PUBLICATION, PUBLICITY AND LIBRARY

The Indian Journal of Malariology is keeping up the tradition and hopes with which it was revived in the year 1981, after 17 years of discontinuation.

Another landmark achieved in publications is the launch of a quarterly magazine in Hindi "Malaria Patrika". It carries articles on various aspects of malariology and is published to take the message to the masses. The response from target audience is very encouraging.

The Centre has also brought out a hard bound publication entitled "Community Participation in Malaria Control". In this book experience on community participation from various areas in the country in relation to vector/malaria control has been reviewed.

Five coloured educational charts for the purpose of display and teaching in health centre, schools, colleges, Medical colleges and institute are printed.

The library of the Centre is catering to the need of its scientists by offering current awareness services, list of new arrivals, inter library loan, citation analysis, user education service and reference service, reprint request and reprographic services. It also offers Medline services through NIC. Library automation has been completed. Reterival can be done through author, journal, year or subject.

SDI services are also being provided on personal request through NIC to the scientific staff of the Centre. Current subscription of foreign journals is 39 and of Indian journals is 7.

The Centre's Library at present has

Books	4656
Reprints	1600
Reports	40
Thesis	13

CONFERENCE ATTENDED AND PAPERS PRESENTED IN 1993

1. Ansari, M.A. Paper presented on ''Domestic Breeding Places of Mosquitoes and their Management''. At the Annual Conference of National Environmental Science Academy and Symposium on ''Environmental Preservation a Role for Everyone,'' Hyderabad. 26-29 December.

2. Atrie, Bharati. Paper presented on ''Cytogenetic Studies of *An. annularis*.'' At the Annual Conference of Indian Society for Malaria and other Communicable Diseases, Delhi. 29-30 December.

3. Biswas, Sukla. Paper presented on ''Detection of Plasmodia Antigen by Enzyme Immunoassay.'' At the Annual Conference of the Indian Society for Malaria and other Communicable Diseases, Delhi. 29-30 December.

4. Chandra, Dinesh. Particpated in DBT sponsored workshop on ''In situ Detection and Analysis of Gene Expression Tissues and Embroys of Drosophila and Mosquito.'' At the Department of Zoology, University of Poona, Pune. 3-24 December.

5. Dhiman, R.C. Attended as observer the ''Regional Working Group Meeting to Implement the Revised Malaria Control Strategy''. At the WHO/SEARO, New Delhi. 22-26 March.

6. Dhiman, R.C. Paper presented on ''Application of Satellite Remote Sensing in Monitoring of Mosquitogenetic Conditions". At the Annual Conference of Indian Society for Malaria and other Communicable Diseases, Delhi. 29-30 December.

7. Joshi, Hema. Participated in TDR/WHO short-term training workshop on ''Molecular Biology of Human Malaria Vectors''. At the Centre for Biotechnology, Anna University, Madras. 1-13 February.

8. Joshi, Hema. Paper presented on ''Genetics Structure of *P. vivax* Isolates in India.'' At the 3rd Asian Congress of Parasitology, CDRI, Lucknow. 18-22 February.

9. Joshi, Hema. Paper presented on ''Genetics Disorders and Malaria.'' At the Annual Conference of Indian Society for Ma-

laria and other Communicable Diseases, Delhi. 29-30 December.

10. Kabilan, Lalitha. Workshop on ''Flowcytometry and Image Analysis''. At the Department of Experimental Medicine, PGIMER, Chandigarh. 29 November-1 December.

11. Kabilan, Lalitha. International symposium on ''HLA in Health and disease : Current states of Art.'' organized by Histocompatibility and immunogenetics unit of AIIMS, New Delhi. 6-8 December.

12. Malhotra, M.S. Attended 5 day advance training course in PC ARC/INFO conducted by International Data Management Ltd. At NISTADS, New Delhi. 13-18 December.

13. Malhotra, M.S. Attended workshop on ''Role of GIS in Pollution Control Studies.'' At NISTADS, New Delhi. 3-7 November.

14. Mittal, P.K., T. Adak and V.P. Sharma. ''Evalution of *Bacillus sphaericus* and *Bacillus thuringiensis israelensis*, Bioinsecticide Preparations for the Control of Mosquito Vector''. Oral presentation at the National Symposium on Pesticides: Future Scenario. At the Indian Agricultural Research Institute, New Delhi. 15-17 April.

15. Mittal, P.K. Paper presented on ''Evalution of Biolarvicide Formulations.'' At the Annual Conference of the Indian Society for Malaria and other Communicable Diseases, Delhi. 29-30 December.

16. Nanda, Nutan. Participated in TDR/WHO short-term training workshop on ''Molecular Biology of Human Malaria Vectors.'' At the Centre for Biotechnology, Anna University, Madras. 1-13 February.

17. Nanda, Nutan. Paper presented on ''Biological Variations among *An. fluviatilis* Sibling Species.'' At the 3rd Asian Congress of Parasitology, CDRI, Lucknow. 18-22 February.

18. Nanda, Nutan. Delivered lecture on ''Recent Advances in Malaria Entomology.'' In Malariaology Training Course, conducted by NMEP, Delhi. March.

19. Nanda, Nutan. Paper presented on ''Cytogenetics and Bionomics of *An. fluviatilis* Sibling Species.'' At the Annual Conference of Indian Society for Malaria and other Communicable Diseases, Delhi. 29-30 December.

20. Raghuvendra, K. Paper presented on ''Biochemical Mechanism of Malathion Resistance in *An. culicifacies*. At the Annual Conference of Indian Society for Malaria and Other Communicable Diseases, Delhi. 29-30 December.

21. Roy, Arati, S. Biswas, M.A. Ansari and Q.B. Saxena. Paper presented on ''Peptide ELISA Enzyme Immunoassay: An Attractive Diagnostic Tool for Malaria Surveillance Study.'' At the XXth Annual Conference of Indian Immunological Society, Bhopal. 17-20 December.

22. Roy, Arati. Paper presented on ''Serology as a Tool to Complement Surveillance''. At the Annual Conference of Indian Society for Malaria and Other Communicable Diseases, Delhi. 29-30 December.

23. Sharma, Arun. ''Bomen FT-IR: Applications, Maintenance and Uses in Protein/Peptide Analysis''. At the Spectroscopy Division of Bhabha Atomic Research Centre, Trombay, Bombay. 8-16 November.

24. Sharma, V.P. Chairman, Malaria section in DBT sponsored SAARC meeting on ''Modern Diagnostics''. At NII, Delhi. 27-29 January.

25. Sharma, V.P. Plenary lecture on "Malaria Situation in India". At the UNIDO course, Anna University, Madras. 1 February.

26. Sharma, V.P. "New Approaches to Malaria Control". II International Symposium on Gentics and Health, Amritsar. 15-18 February.

27. Sharma, V.P. Lecture on "Transmission Dynamics of Malaria". International Symposium in Parasitology, CDRI, Lucknow. 18-21 February.

28. Sharma, V.P. Tropical Diseases; Lead Speaker on "Transmission Dynamics of Malaria". Transgene, Hyderabad. 22 February.

29. Sharma,V.P. Delivered lecture on ''Neem in Malaria Control''. At the World Neem Conference, Bangalore. 24-28 Februrary.

30. Sharma,V.P. Temporary adviser and presented papers on (i) Cost-effectiveness Analysis in Malaria Control and (ii) Technical and Organizational Aspects in Malaria Control Research. Global Malaria Control Strategy meeting in WHO (SEARO). 22-26 March.

31. Sharma, V.P. Member, attended PEEM Steering Committee Meeting in UNCHS, Nairobi. 20-23 April.

32. Sharma,V.P. Presented paper on ''Health Aspects and Water related Diseases due to Water Supply and Irrigation River Valley Projects''. At the National Round Table Discussion on "Environmental Impacts of Water Resources Development", organized by Institution of Engineers (India), Delhi. 4-5 June.

33. Sharma,V.P. Delivered several lectures. At the nine week Malariology Course for the DPHOs of Nepal, Delhi. 12 April-12 June.

34. Sharma,V.P. Temporary adviser, attended ''Applied Field Research (CTD) meeting'' in WHO, Geneva. 26-28 May.

35. Sharma,V.P. Key note address on ''Environmental Management of Malaria''. At the M.S. Swaminathan Research Foundation, Madras. 12 August.

36. Sharma,V.P. Meeting on the ''Identification of Research Priorities in Science and Technology'', At Rajiv Gandhi Foundation, Delhi. 13 August.

37. Sharma,V.P. Delivered keynote address on "Problem of malaria in India with particular reference to urban malaria". At the seminar on malaria in GS Medical College, and KEM Hospital, Bombay. 22 August.

38. Sharma,V.P. Short-term consultant (STC, WHO) Bangladesh. 3 September - 16 October.

39. Sharma,V.P. Member, ''Study Group on Vector Biology'', WHO, Geneva. 16-24 November.

40. Sharma,V.P. Chairman of a Session. At the Science and Law Symposium, Delhi. 17-19 December.

41. Subbarao, Sarala K. Faculty member and delivered lectures and conducted demonstrations on ''Malaria Vector and Spe-

cies Complexes with Special Reference to S.E. Asia. Importance of Sibling Species in Malaria Epidemiology'' and ''Methods used in the Detection of Species Complexes. Their Scope and the Limitations''. At the WHO/TDR short-term training workshop on ''Molecular Biology of Human Malaria Vectors'', Centre for Biotechnology, Anna University, Madras. 1-13 February.

42. Subbarao, Sarala K. Consultant and papers presented on ''Indian Sub-continent Anophelines and the Malaria Problem on a Regional Basis'' and ''The Phenomenon of Cytoplasmic Incompatibility in Mosquitoes''. At the joint FAO/IAEA group meeting on ''The Potential for Genetic Control of Malaria-transmitting Mosquitoes, Vienna. 26-30 April.

43. Subbarao, Sarala, K. Faculty member, delivered lectures and conducted demonstrations on ''Anopheline Biology and the role of Sibling Species in Malaria Epidemiology'', ''Tools for the Identification of Sibling Species'' and ''Endosymbionts and their Role in Cytoplasmic Incompatibility''. At the DBT sponsored short-term training course on ''*In situ* Detection and Analysis of Gene Expression in Tissues and Embryos of Drosophila and Mosquito'', Department of Zoology, University of Poona, Pune. 3-24 December.

44. Subbarao, Sarala K. Delivered lecture on''Recent Advances in Malaria Entomology''. At the Malariology Training Course of NMEP, Delhi. December.

45. Valecha, Neena. Guest lecture on ''Complications of Malaria: An Update on Malaria''. At the seminar organised by the Indian Society of Paediatrics, Surat. 21 March.

46. Valecha, Neena. Participated in seminar on ''Toxicology Status, Trends and Prospects''. At the India International Centre, Delhi. 31 March.

47. Valecha, Neena. Lecture on ''Severe and Complicated Malaria''. At the workshop for Medical Specialists/ Engineers, Ministry of Defence, New Delhi. 20 July.

48. Valecha, Neena. Lecture on ''Chemotherapy of Malaria.'' At the workshop on the control of malaria for Army Medical Corps and Engineers. At 115 Base Hospital, Gauhati. 13-15 September.

49. Valecha, Neena. Lecture on ''Chemotherapy of Malaria''. At the workshop for Medical Specialists/ Engineers. Mininsty of Defence, Agra Airforce Station, Agra. 19-20 October.

PAPERS PUBLISHED IN 1993

1. Adak, T., Sarala K. Subbarao and V.P. Sharma. Inheritance and linkage of malic enzyme in *Anopheles stephensi*. *J. Amer. Mosq. Contr. Assoc.*, **9**(3): 313-315.

2. Ansari, M.A. Innovative methods to repel the mosquitoes. *NESA News letter*, **12**: 2-4.

3. Bhalwar, Rajvir, T. Adak, C.P. Batra and V.W. Tilak. Evaluation of new method of mosquito control in the armed forces field trial with *Bacillus sphaericus*. *Medical Journal of Armed Forces India*, **49**: 57-60.

4. Bhatt, R.M., R.C. Sharma, H.C. Srivastava, A.S. Gautam and D.K. Gupta. Interspecific associations among Anophelines in different breeding habitats of Kheda district, Gujarat : Part II – non-canal area. *Indian J. Malariol.*, **30**(2): 91-100.

5. Chand, S.K., R.S. Yadav and V.P. Sharma. Seasonality of indoor resting mosquitoes in a broken-forest ecosystem of north-western Orissa. *Indian J. Malariol.*, **30**(3): 145-154.

6. Dua V.K., S.K. Sharma and V.P. Sharma. Application of Bactoculicide (*Bacillus thuringiensis* H-14) for controlling mosquito breeding in industrial scrap at BHEL, Hardwar (U.P.). *Indian J. Malariol.*, **30**(1): 17-21.

7. Dua, V.K., Paritosh K. Kar, Suresh Kumar and V.P. Sharma. *In vivo* and *in vitro* sensitivity of *Plasmodium falciparum* to chloroquine at Indian Oil Corporation, Mathura (U.P.). *Indian J. Malariol.*, **30**(1): 29-35.

8. Dua, V.K., Reema Sarin and Anil Prakash. Determination of quinine in serum plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high performance liquid chromatography. *J. Chromatography*, **614**: 67-93.

9. Haq, S., H. Prasad, R.N. Prasad and T. Sharma. Availability and utility of local fishes of Shahjahanpur for mosquito control. *Indian J. Malariol.*, **30**(1): 1-8.

10. Kumari, Roop, Hema Joshi, A. Giri and V.P. Sharma. Feeding preferences of *Anopheles sundaicus* in Car Nicobar Island. *Indian J. Malariol.*, **30**(4): 201-206.

11. Mittal, P.K., T. Adak and V.P. Sharma. Effect of temperature on toxicity of two bioinsecticides Spherix (*Bacillus sphaericus*) and Bactoculicide (*Bacillus thuringiensis*) against larvae of four vector mosquitoes. *Indian J. Malariol.*, **30**(1): 37-44.

12. Mittal, P.K., T. Adak, C.P. Batra and V.P. Sharma. Laboratory and field evaluation of Spherix a formulation of *Bacillus sphaericus* (B-101), to control breeding of *An. stephensi* and *Cx. quinquefasciatus*. *Indian J. Malariol.*, **30**(2): 81-90.

13. Pant, C.S., D.K. Gupta, R.M. Bhatt, A.S. Gautam and R.C. Sharma. Three genetic markers and malaria in upper caste Hindus of Kheda District of Gujarat State. *Indian J. Malariol.*, **30**(4): 229-233.

14. Pillai, C.R. and N.N. Singh. Role of macrophages in experimental malaria: I. Development of immunobioassay indicators. *Indian J. Malariol.*, **30**(1): 23-28.

15. Prasad, H., R.N. Prasad and S. Haq. Control of mosquito breeding through *Gambusia affinis* in rice fields. *Indian J. Malariol.*, **30**(2): 57-65.

16. Prasad, R.N., M.K. Das, T. Sharma and G.D.P. Dutta. Prevalence of filariasis in rural areas of Shahjahanpur district (U.P.). *Indian J. Med. Res.*, **97**(A): 112-114.

17. Sarin, Kumud, Ajay Kumar, Anil Prakash and Arun Sharma. Oxidative stress and antioxidant defence mechanism in *Plasmodium vivax* malaria before and after chloroquine treatment. *Indian J. Malariol.*, **30**(3): 127-134.

18. Sharma, Arun. Subcellular distribution of superoxide dismutase and catalase in human parasite *Plasmodium vivax*. *Indian J. Expt. Biol.*, **31**: 275-277.

19. Sharma, S.N., S.K. Subbarao, D.S. Choudhury and K.C. Pandey. Role of *An. culicifacies* and *An. stephensi* in malaria transmission in urban Delhi. *Indian J. Malariol.*, **30**(3): 155-168.

20. Sharma, V.P., B.N. Nagpal and Aruna Srivastava. Effectiveness of neem oil mats in repelling mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.*, **87**: 626.

21. Sharma, V.P., B.N. Nagpal, Aruna Srivastava and Anoop Rawal Indian Anopheles fauna and species distribution information system. *Mosq. Syst.*, **25**: 64-65.

22. Sharma, V.P., M.A. Ansari and R.K. Razdan. Use of kerosene lamp containing synthetic pyrethroids to repel mosquitoes. *Indian J. Malariol.*, **30**(3): 169-176.

23. Sharma, V.P. Malaria control. *Neem Research and Development*. Eds. N.S. Randhawa and B.S. Parmar, Publication No. 3 (Society of Pesticide Science, India): 235-241.

24. Sharma, V.P. and R.C. Dhiman. Neem oil as a sandfly (Diptera: Psychodidae) repellent. *J. Amer. Mosq. Contr. Assoc.*, 9(3): 364-366.

25. Sharma, V.P. Ecosystem approach to malaria control. Eds. V.P. Sharma and Om Prasad. In *Proceedings of the National Academy of Sciences* 63(B), India.: 47-55.

26. Singh Neeru, A.K.Mishra and O.P. Singh. Preliminary observations on mosquito collections by light traps in tribal villages of Madhya Pradesh. *Indian J. Malariol.*, **30**(2): 103-108.

27. Subbarao, Sarala, K., Nutan Nanda, R.K. Chandrahas and V.P. Sharma. *Anopheles culicifacies* complex: Cytogenetic characterization of Rameshwaram Island populations. *J. Amer. Mosq. Contr. Assoc.*, **9**(1): 27-31

28. Subbarao, Sarala K., Nutan Nanda, K. Vasantha, V.K. Dua, M.S. Malhotra, R.S. Yadav and V.P. Sharma. Cytogenetic evidence for three sibling species in *Anopheles* (Diptera: Culicidae). *Ann. Entomol. Soc. Amer.*, **87**(1): 116-121.

29. Thavaselvam, D., Ashwani Kumar and P.K. Sumodan. Insecticide susceptibility status of *Anopheles stephensi*, *Culex quinquefasciatus* and *Ae. aegypti* in Panaji, Goa. *Indian J. Malariol.*, **30**(2): 75-79.

30. Tripathi, K.D., A.K. Sharma, N. Valecha and S. Biswas. *In vitro* activity of fluoroquinolones against chloroquine-sensitive

and chloroquine-resistant *Plasmodium falciparum*. *Indian J. Malariol.*, **30**(2): 67-73.

31. Tripathi, K.D., A.K. Sharma, and N. Valecha. Norfloxacin in treatment of vivax malaria. *Med. Sci. Res.*, **21**: 159-160.

32. Tripathi, K.D., A.K. Sharma, and N. Valecha. Curative efficacy of norfloxacin in falciparum malaria. *Indian. J. Med. Res.*, **97**: 176-178.

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